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GENETICAL STUDIES ON THE CHLOROPHYLL APPARATUS IN OATS AND WHEAT

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INTRODUCTION.

ALL reports on chlorophyll aberrations of oats and wheat have established an exceedingly low frequency of spontaneous chlorophyll changes especially in the high-polyploid oats and wheats. Even cases of obviously non-Mendelian chlorophyll aberrations are apparently scarce in these polyploid cereals, as shown by the survey in Chapter I. Not only the polyploid oats and wheats, however, are characterized by this seemingly great stability of the numerous genes responsible for the development of a normal chlorophyll apparatus. Even in the diploids (the taxonomy of oats follows MALZEW, 1930, that of wheat PERCIVAL, 1921), e. g., *Avena strigosa* SCHREB., *A. brevis* (ROTH) THELL. and *Triticum monococcum* L., *T. aegilopoides* BALF., and the tetraploids, e. g., *Avena barbata* (POTT) THELL., *A. abyssinica* (HOCHST.) THELL. and *Triticum durum* DESF., *T. turgidum* L., *T. dicoccum* SCHÜBL., spontaneous chlorophyll aberrations are but infrequently reported. Quite other frequencies of such aberrations have, however, been met with in the investigations on diploid barley. Here a great many spontaneous chlorophyll changes have been described in the past. For literature surveys up to 1932—1933 the reader is referred to DE HAAN's monograph (1933), and after that date to GUSTAFSSON (1938, 1940). An immensely raised chlorophyll mutation frequency — presumably up to 5.000 times — has also been obtained in barley by X-ray treatment.

Since chlorophyll changes are as a rule comparatively easy to detect, it is not astonishing that the obvious difference in the frequency of these aberrations between barley, on the one hand, and wheat and oats on the other, has attracted the interest of both the practical breeder and the theoretical geneticist, from several points of view. The extremely scarce occurrence of spontaneous chlorophyll aberrations in wheat and oats, perhaps most clearly shown by ÅKERMAN (1922, 1929; ÅKERMAN and MÜHLOW, 1933; ÅKERMAN and FRÖIER, 1941), on the basis of

investigations at Svalöf for more than 20 years, manifests itself not only in comparison with barley. Also other diploids, such as *Pisum*, *Secale*, *Zea*, *Antirrhinum* and other thoroughly investigated plants, show a considerable frequency of spontaneous changes affecting the chlorophyll development.

By means of irradiation, STADLER (1929) directly compared the chlorophyll mutation frequencies of *Hordeum vulgare* (2n = 14) with those of *Avena brevis*, *A. strigosa* (2n = 14), *A. byzantina* (KOCH) THELL., *A. sativa* (2n = 42), *Triticum monococcum* (2n = 14), *T. dicoccum*, *T. durum* (2n = 28), and *T. vulgare* (2n = 42). He found a certain number of chlorophyll mutations in the diploid oats, the diploid and tetraploid wheats. In this comparison no mutations were found in irradiated hexaploid oats and wheat. Although the number of treated seeds was limited, STADLER concluded that the low frequency of induced chlorophyll mutations in polyploids was due to gene reduplication. He emphasizes, however, that other circumstances may also be responsible for the low mutation frequency. Evidently, the proportion of non-reduplicated genes, according to STADLER, is very small in the hexaploid species. As regards the tetraploids, he states that »a considerable proportion of the mutable genes appear to be dominant in only one of the two chromosome groups» (l. c., p. 881), since several mutations were found in these tetraploids (only wheat tetraploids were studied).

As for the direct comparison with barley, STADLER mentions that an X-ray treatment producing 40 mutations in barley produced merely a single recessive albino from the hexaploid oat variety Kherson (*A. sativa*). In an experiment published the following year (STADLER, 1930) the same X-ray treatment did not produce any chlorophyll mutation at all either in the winter wheat Harvest Queen or in the spring wheat Marquis (both *T. vulgare* varieties).

STADLER moreover predicts: »If this hypothesis is correct, the species of *Avena* and *Triticum* with 7 pairs of chromosomes should mutate at rates comparable with those of common barley» (1930, p. 11). The fulfilment of this prediction has now been practically achieved, partly by the work of L. SMITH (1936, 1939) on *Triticum monococcum* and *T. aegilopoides* (both diploids), partly by the present writer on *T. monococcum* (pp. 379 ff.), *Avena strigosa* (pp. 364 ff.) and — to a smaller extent — also on *Avena brevis* (pp. 371 ff.), the induced mutation rate of barley being at present fairly well known by STADLER's own experiments (1928, 1929, 1930, 1931), but also through GUSTAFSSON's irradiation work of the last few years.

STADLER's general view regarding the causes of the great stability of the chlorophyll apparatus in the polyploid cereals can no doubt serve as a basis for all further research work on the subject. It is interesting to find that similar theories on gene reduplication in plants were ventilated in the discussions on polyploidy in the same year as that in which STADLER published his results. HUSKINS (1929) referred to the classical cases of NILSSON-EHLE (1909) of wheat varieties with one, two or three factors for red grain colour and of oats with up to three factors for grain colour and at least three[1] for ligule development, together with the interesting case of ÅKERMAN's (1922) three-factorial *lutescens* oat chlorophyll aberration. HUSKINS contrasts these facts with »the situation in barley or rye in which duplicate factors are relatively rare and single factor differences are common» (1929, p. 29). After stating that duplicate factors can, of course, arise even in a diploid, either through parallel gene mutation in different chromosomes or through duplication of parts of chromosomes, HUSKINS finds that »the simplest and most plausible explanation of the common occurrence of duplicate factors in tetraploids and triplicate factors in hexaploids is that they were present in different original diploid species and have been brought together in the cultivated cereals by allopolyploidy» (l. c., p. 29). HUSKIN's opinion is strongly supported by certain cases of homologous, polymeric chlorophyll factors, such as the *lutescens* factors in oats (ÅKERMAN, 1922; ÅKERMAN and FRÖIER, 1942). (On the use of the term »homologous, polymeric factors», cf. ÅKERMAN and FRÖIER, 1941, pp. 397—398.)

The view regarding a general connection between the number of homologous, polymeric factors and the degree of polyploidy has afterwards been repeatedly discussed. Thus, among other authors, MÜNTZING (1937, p. 398) on investigating the dimeric segregation of the genes for hairiness in the tetraploid *Galeopsis Tetrahit*, reconsiders the most obvious cases of polymery in polyploids (NILSSON-EHLE, 1909; ÅKERMAN, 1922; MEURMAN, 1926; J. CLAUSEN, 1926 and 1931; ÅKERMAN and MÜHLOW, 1933) and states »that it is certainly not simply a chance occurrence that the most typical cases of polymeric factors have been found in polyploid species».

[1] NILSSON-EHLE (1909) reported four factors for ligule development in oats; ÅKERMAN and MÜHLOW (1933), however, repeated the same cross experiments on a large scale and found only three factors. This note has been added, as MATSUURA, in his second edition of the bibliographical monograph on plant genetics (1933, p. 40), tabulates four ligule factors — as the only known case of tetragenic inheritance in *Avena*.

SCOPE OF INVESTIGATION.

(1) Prompted by the reports by ÅKERMAN (1922, 1929) on the genetics of the chlorophyll aberrations *lutescens* and *chlorina* in hexaploid oats, the present writer decided to make a closer study of these forms, mainly for the purpose of elucidating the factorial distribution in various oat strains. This work, though involving laborious routine research, should, nevertheless, reveal possible non-visible mutation steps. STADLER (1930) assumes the existence of at least »some genes in the *aaA* condition, as a result of previous mutations occurring in the course of the past evolution of the species» (l. c., p. 10). As the correctness of this assumption could be tested in a somewhat extensive factorial distribution study, several oats were subjected to such an analysis. Certain results had already been published at an earlier date (ÅKERMAN and FRÖIER, 1941, on *chlorina*, and the same authors 1942 on *lutescens*).

(2) As, in the course of the last few years, facilities for carrying out experiments with X-ray induction of mutations had been granted to the Swedish Seed Association, the author decided to investigate (a) the diploid species *Avena strigosa*, *A. brevis* and *Triticum monococcum* and to compare the mutation frequencies obtained with those previously observed in barley. (b) X-ray treatment of certain tetraploid and hexaploid oats and wheats was planned in order to elucidate the possibility of inducing any visible chlorophyll mutations at all in these polyploids. Secondly, the results might give indications as to the amount and types of chlorophyll genes in the monofactorial condition (i. e. as *aA* and *aaA* in tetra- and hexaploids).

(3) The study of certain spontaneously arisen chlorophyll aberrations of both diploid and hexaploid oat strains was continued.

(4) A simple, but satisfactory classification of the chlorophyll mutations procured was planned. Comparisons with earlier classifications were considered desirable, especially with the system of GUSTAFSSON (1938, 1940) for the taxonomy of barley chlorophyll mutations. Some anatomical and physiological observations on the mutant forms were undertaken, in order, if possible, to facilitate the classification. In this respect, many restrictions were found necessary, since a number of specific problems arise which need special assistance from a qualified physiologist.

(5) A more detailed knowledge of the genetical and cytological effects of the various X-ray treatments was further contemplated. In the present work, however, solely genetical results have been considered. A number of necessary cytological investigations on induced mutations

will therefore be completed and published later. Moreover, not all mutations detected could be adequately studied even genetically. A study on the degree of cytological disturbances following different X-ray dosages in five species of wheat and oats has, however, been previously published (FRÖIER, GELIN and GUSTAFSSON, 1941; FRÖIER, GUSTAFSSON and TEDIN, 1942).

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I. PREVIOUS RESEARCH WORK ON GENETICALLY CONDITIONED CHLOROPHYLL ABERRATIONS OF OATS AND WHEAT. A CRITICAL SURVEY.

1. OATS, MENDELIAN INHERITANCE.

(1) Beginning with oats, we meet with the statement of D. C. SMITH (1938) that »ZHEGALOV, cited by DE HAAN, appears to have been the first to observe *albino* seedlings in the genus *Avena*» (l. c., p. 64). Before the work of ZHEGALOV (1920), however, we find that as far back as 1913 it had been stated by NILSSON-EHLE that he had observed »ganz vereinzelte weisse Pflanzen» in three varieties of hexaploid oats of different origin (l. c., p. 299). NILSSON-EHLE did not closely investigate these albinotic oat plants. The report of D. C. SMITH thus has to be corrected in respect to the priority of first known chlorophyll-deficient oat forms. The mistake may be attributed to DE HAAN's

monograph (1933), where (on p. 360) NILSSON-EHLE's statement regarding albinotic oat plants has not found place in the tabulation, whilst on the next page NILSSON-EHLE's work of 1913 is cited — here, however, with reference to the genus *Hordeum*. In another literature review on the subject PHILP (1935) clearly states that NILSSON-EHLE was the first to report any chlorophyll-deficiencies in oats.

(2) The second reported case of oat chlorophyll aberration was published by ZHEGALOV (1920). Also on this occasion *albinotic* seedlings were found. As the present author has been unable to procure either the original work or any review of ZHEGALOV's paper, it cannot be subjected to criticism. DE HAAN (1933) refers to these albinotic seedlings (l. c., p. 360) under »Chapter I. A. § 1.», i. e. as unicoloured types (not variegated), Mendelian inherited. Apparently no plastid inheritance was assumed here.

(3) The third report on chlorophyll-deficient oats was that published by ÅKERMAN (1922) in his paper on the *lutescens* strain procured from crosses between central-Swedish black-hulled oats and Probsteier oats of Danish origin (Abeds Nova). Three polymeric, homologous (most probably identical) factors, L_s, L_p and L_n, were found to act in the parental strains, L_s in the former parent, L_p and L_n in the latter. The last-mentioned statement was made by ÅKERMAN and FRÖIER (1942).

(4) The fourth report on chlorophyll changes in hexaploid oats was published by ÅKERMAN (1929). In Guldregn I oats he discovered a mutant type, the f. *chlorina*. This form, too, proved to be trimerically inherited. Some oat strains were investigated by means of cross analysis, and were shown to possess one, two or three *chlorina* factors. They are all completely recessive in respect of normal green colour. It was ascertained that the f. *chlorina* had been produced by mutation from the monofactorial mother strain. Thus, successive steps of mutation could be traced from trifactorial down to monofactorial stages of certain investigated oat strains. The *chlorina* recessives readily set seed and can be propagated with comparative ease; the principal descriptions have been given by ÅKERMAN and FRÖIER (1941).

(5) The fifth report on the same subject is found in a cyto-genetic study by PHILP (1935). In crosses *Avena sativa gigantica* × *A. fatua* PHILP obtained F_4 progenies segregating into about 1 green and 6 *albina* plants. The green plants had 41 chromosomes, and the albinos were short of two chromosomes, i. e. possessed only 40. Abnormal chromosome pairing (allosyndesis) was observed in the hybrid deriva-

tives. At least two independent factors were shown to control the production of chlorophyll. PHILP is inclined to assume even three polymeric factors on the basis of the three chromosome sets in the allopolyploid oats investigated. He also stated that chromosome loss following allosyndesis revealed previous recessive mutations in polymeric factors for chlorophyll production in the allopolyploid parental species. The chief conclusions drawn from PHILP's very exact studies were the following: (1) polymeric factors control chlorophyll production in hexaploid oats, (2) these pairs of factors lie in homologous chromosomes of different sets which do not normally pair, (3) abnormal pairing of these chromosomes in the hybrids results in segregation of pure recessive (*albino*) types, (4) abnormalities in the inheritance of these segregated characters are due to (a) loss of chromosomes, (b) crossing-over between the dissimilar homologues following their exceptional pairing. PHILP (l. c., p. 291) also briefly mentions a *viridoalbina* wheat mutation, found by NEATBY (1933), and adds: »Forms similar in appearance to this mutant were observed by the present author in F_3 families of the cross *A. fatua* × *A. sativa gigantica*, and in one F_4 family from the cross *A. sativa* var. Banner × *A. fatua*».

(6) The sixth case of chlorophyll-deficient oats was published by D. C. SMITH (1938) in connection with his studies on oat seedling resistance to stem and crown rusts. Roughly 7.000 pots of plants belonging to several oat cross progenies, each pot containing 20—40 individuals, were studied. One pot of the variety Victoria contained 29 plants, 8 of which were typical *albina* plants. 78 additional seedlings from the same plant consisted of 65 normal green and 13 albinos. SMITH thus found altogether 86 green to 21 albinos, which data suggest a ratio of three normal to one albino.

Although SMITH himself points out the extremely rare occurrence of such oat chlorophyll deficiencies and has given a fairly comprehensive review of the reports in the literature in regard to such abnormalities, he unfortunately had to abandon further investigation of the discovered form. Thus, progeny tests of the normal plants in this single segregating progeny among 7.000 were not undertaken. It would have been of great interest to ascertain whether he was really concerned here with a case of spontaneous mutation, or whether the appearance of the aberrant form might have been due to spontaneous crossing. The initial plant of this segregating progeny seems to have been entirely green. The possibility of spontaneous mutation must here be left open. On the other hand, it is quite conceivable that spontaneous crossing may have occurred; the only way to rule out this possibility would have been a detailed investigation of the next generation from the segregating progeny, in order to establish its complete homogeneity and identity with the mother variety Victoria in observable properties. A segregation into several such characters would most probably have been found as result from a supposed spontaneous crossing. Against the possibility of a spontaneous cross, however, the objection can be raised that the ratio 3 : 1 is not primarily to be expected as the result of such a cross. The ratios

15 : 1 or 63 : 1 are to be expected in the first place, in view of our present accumulated knowledge of polymeric factors governing chlorophyll formation in cereal polyploids. It is still an open question whether any typical *albina* has hitherto been produced in hexaploid oats by spontaneous mutation.

(7) The seventh case was first published merely in the form of a preliminary note (ÅKERMAN and FRÖIER, 1941, p. 372) on the f. *albovirescens*, found by FRÖIER in 1939. This aberration, undoubtedly the result of spontaneous mutation, is a typical representative of the *virescens* group (DE HAAN's scheme, 1933, p. 364) or subgroup *viridoalbina* (chief group: *alboviridis*) according to GUSTAFSSON, 1940, p. 4. The new form was found in the progeny from a cross involving primarily four different ancestral strains (Ch. IV).

2. OATS, NON-MENDELIAN INHERITANCE.

In connection with these seven cases of chiefly Mendelian chlorophyll aberrations in oats, arisen as spontaneous mutations or segregated from cross progenies (X-ray induced mutations are not included in this review, but will be referred to in the subsequent discussion) six recorded cases of such aberrations exhibiting non-Mendelian or complex inheritance must be mentioned:

(1) CHRISTIE (1921) found in a single plant in the Norwegian oats Møistads Grenadier yellow-striping on leaves and glumes. He interpreted the case with an assumption (l. c., p. 140), »dass man hier eine nichtmendelnde Verebung hat, entsprechend was BAUR, CORRENS, IKENO und MILES früher für andere Pflanzenarten gefunden haben». It is somewhat astonishing to find that DE HAAN (1933, p. 369) afterwards classifies CHRISTIE's results as a case of »variegated chlorophyll-deficiencies as a result of mutable genes», this mode of inheritance having been first observed by CORRENS in *Mirabilis Jalapa* (1908—1909, 1910). The studies, however, were not continued far enough to rule out a possible explanation as a case of plastid inheritance, like that given by ÅKERMAN (1933) in regard to his *luteostriata* oat form; see below.

(2) COFFMAN, PARKER and QUISENBERRY (1925) noted white seedlings in the progeny of a whitish-striped plant from the variety Burt oats (*Avena byzantina*). In the next generation »about 800 plants were grown, of which a considerable percentage exhibited the chlorotic condition in varying degrees, ranging from only a few rather indistinct longitudinal yellowish-white stripes to the apparent total absence of chlorophyll» (l. c., p. 57). The yellowish-white striping thus found must probably be attributed to plastid inheritance with unequal random distribution of plastids.

(3) FERDINANDSEN and WINGE (1930) describe a characteristic spot necrosis, »blotch leaf», in a Norwegian oat of Snaasen type, originating from Nordfjord. The disease includes destruction of chlorophyll at the necrotic spots. Cross experiments with Seger oats showed the necrosis to be of genetic nature. The disease had not been caused by any parasite or virus infection. »The segregations in F_2 and following generations are greatly varying and discordant to Mendelian numerical relations, for which reason the hereditary element in question cannot be associated with the chromosomes. The hereditary element, which produces blotch leaf, is with the greatest probability to be regarded as associated with the plastids of the variety» (l. c., pp. 175—176). Blotch leaf is most easily transmitted through eggs, but also through pollen, to following generations. The reddish-brown margins of the necrotic spots make this genetically produced disease or abnormality very characteristic. The authors place their results in connection with EMERSON's investigations on an almost identical form of blotch leaf in maize (EMERSON, 1923) and state that EMERSON's interpretation of blotch leaf showing simple Mendelian segregation, but disguised by incomplete relations of dominance, cannot be upheld. »Blotching», as an oat chlorophyll aberration, is not known in this form in any other case; but in an interesting Mendelian inherited *tigrina* mutation of *Avena strigosa* ($2n = 14$) the present writer has found transverse bands or dots with blue-red-brownish necrotic margins, in part closely resembling those on the blotch leaf necrotic spots (Chapter IV).

(4) ÅKERMAN (1933) found in Guldregn I oats a yellowish-striped plant, a f. *luteostriata*, which was studied for six subsequent generations, involving reciprocal crosses with the mother strain. The results left no doubt as to plastid inheritance exclusively through the mother parent.

A whitish-striped oat plant was also found in a progeny from a cross Kron oats × Strube's Schlanstedter; owing to the limited number of its offspring its mode of inheritance could not be ascertained. In succeeding years ÅKERMAN (1933, p. 269) reports numerous cases of whitish-striped and yellowish-striped oat plants. Among his observations one new yellowish-striped Guldregn I oat plant was studied more thoroughly. The results were identical with those obtained with the first investigated f. *luteostriata*. Also the present writer has on three occasions observed yellowish-striped forms in hexaploid oat strains.

Since two of these three cases again happened to occur in Guldregn I oats, there is some reason to suppose that this old pure oat line shows comparatively frequent plastid aberration. In preliminary experiments 1938—1939 with production of mutations by means of heat treatment (not published), the author, prompted by

the works of SCHKWARNIKOW (1936 a and b), KIRNOSSOWA (1936), SCHKWARNIKOW and NAWASHIN (1934), CARTLEDGE, BARTON and BLAKESLEE (1936), and PETO (1933), tried to study the action of temperature and hygroscopic conditions on dormant seeds of several oats. 2.000 kernels of the variety tested were exposed to 75° C. for 100 minutes in a thermostat, 2.000 kernels to 85° C., and, finally, 2.000 kernels to 96° C. Four varieties of hexaploid oats were tested: Seger, Guldregn I, Engelbrekt II and Orion II. Among the plants from the treated seeds a number of chimaeras appeared, yellow-striated and also types more or less yellow-tinged. All chimaeric plants found afterwards, however, changed to normal colour. GOLDSCHMIDT (1935) found in *Drosophila* similar, non-inherited modifications after heat treatment. On account of their similarity to the phenotype of certain mutations, he called these modifications »phenocopies». FRIESEN (1935) speaks of the same type of modification under the name of »morphoses». Now, in the author's above-mentioned experiments, Guldregn I proved definitively more sensitive in respect to heat treatment than the other three varieties: the amount of chlorophyll-aberrant phenocopies was 5—12 times larger in Guldregn I than in the other oats. Although the experiments were by no means conclusive, as only 4 oat varieties were used, they may be mentioned as a basis for further experiments; the general results even from the X-ray experiments at Svalöf indubitably support the view of Guldregn I as a hexaploid oat, obviously sensitive to spontaneous and induced chlorophyll disturbances. In the variety Guldregn II, derived from the cross Seger × Guldregn I (ÅKERMAN, 1928), the author has in vain tried to find spontaneous or induced chlorophyll aberrations of the *albostriata* or *luteostriata* types. Guldregn II has, however, given rise to a *luteomaculata* type (with yellow-dotted leaves), described in Chapter IV.

(5) In the same year ROBB (1933), independently and without knowledge of ÅKERMAN's results, reported yellowish-striped oat plants from the cross Sandy × Record. He showed that the transmission of the striped-leaf character occurred by the female parent solely. The widely different segregation ratios found and the variation in striping on different tillers of the same plant furnished evidence in support of the view that the occurrence of striping depends on the character of the plastids.

(6) LOVE and CRAIG (1936) reported yellowish-striped plants in cross progenies from the combination Ruakura (hexa- or tetraploid variety?) × a strain of *Avena sterilis macrocarpa*. In the second generation they found 17 striped plants out of a total of 386, which suggested approximately a 15 : 1 ratio. Further results obtained from crosses with normal green plants and the reciprocal cross, however, showed »that the behaviour of the chlorophyll deficiency is not to be explained as due to the chromosomes, but is apparently cytoplasmic in nature» (l. c., p. 1011). It is not clear whether the writers mean plasmon action in the sense of CORRENS (1928) — as, e. g., in DAHLGREN's *Geranium bohemicum* × *deprehensum* cross; DAHLGREN, 1923, 1925 — or attribute the conditions to action of unequally distributed plastids. From the data reported by LOVE and CRAIG it seems most probable that the latter explanation — in complete accordance with ÅKERMAN's conclusions in regard to the *luteostriata* oat form (1933) — is correct. The yellow-striping was transmitted exclusively by the female parent.

3. WHEAT, MENDELIAN INHERITANCE.

Turning to wheat, we find, as in oats, a very limited number of reported chlorophyll deficiencies. Owing to lack of access to the original papers some of them can be recorded here merely in the briefest form. Others will be more thoroughly considered. The following is a review of the reports on the subject, arranged in chronological order.

(1) In 1922 MEISTER (cited by PHILP, 1935, p. 291) reports that crosses between tetraploid and hexaploid wheats have often given *albinos* and *chlorotic* forms. MEISTER worked chiefly with hybrids between *Triticum durum* and *T. vulgare*. It has not as yet been found possible to obtain further particulars regarding MEISTER's report. The facts gathered by him, however, can easily be brought into correspondence with those of most of the authors cited below.

(2) In 1925 VAVILOV and JAKUSHKINA — in their work on the new tetraploid wheat species *Triticum persicum* VAV. — arrive at the same conclusion as MEISTER regarding crosses between tetraploid and hexaploid wheats. They thus report (l. c., p. 145): »Very often in crosses between the species of these two groups the occurrence of *albinos* is observed. In a cross between soft wheat (*T. vulgare* v. *lutescens* AL.) and West-European emmer *T. dicoccum* v. *pycnurum* in 17 families out of 51 there were plants more or less devoid of chlorophyll. *Albinos* were also observed in a cross of *T. durum* v. *melanopus* AL. from Persia with *T. Spelta* No. 25, when the whole first generation proved to be albinos and perished. Rarely such hybrids were observed at the Saratov Experiment Station among hybrids of crosses between soft wheat and durum wheat.«

(3) HARRINGTON and SMITH (1928) reported from a cross between two varieties (Khapli and Early Emmer), both of *Triticum dicoccum* (tetraploid), that approximately $^1/_{16}$ of the seedlings were yellow and lacked chlorophyll. Each of the parents must have carried one recessive gene for inhibition of chlorophyll development. But, as they carried also an epistatically acting gene for normal chlorophyll, the presence of the recessive gene was not apparent. To judge from descriptions left by the writers, the yellow seedlings must be classified as *xantha* forms (DE HAAN, 1933; GUSTAFSSON, 1940).

(4) KHINTSHUK (1929), working on the genetics of *Triticum Timopheevi* ZHUK., crossed the tetraploid species *T. Timopheevi* ZHUK. var. *viticulosum* ZHUK. with *T. persicum* VAV. He then observed in F_3 and F_4 chlorophyll defects of the *albina*, *xantha* and *flavescens* types. It is not quite clear from KHINTSHUK's original paper whether he regards his

results as conclusive for the existence of dimeric ratios, as in HARRINGTON and SMITH's above-mentioned investigation, or not. He concludes that the genetical remoteness of *T. Timopheevi* from the other 28-chromosome wheats is confined by segregation data according to separate features: »The feature of segregation according to colour of the leaves, with the appearance of plants defective in chlorophyll, of the types *albina*, *xantha* and *flavescens*, demonstrating the difference in not less than two principal chlorophyll factors in the crossed forms» (l. c., p. 652). In the paper it is further stated that similar *albina* forms have been met with in crosses between 42 and 28 chromosome wheats (*T. sphaerococcum* — 2n = 42 — and *T. durum*, *turgidum* and *persicum* — 2n = 28). In the crosses of *T. persicum* × *T. Timopheevi*, *T. dicoccum* × *T. vulgare* as well as in all crosses with *T. sphaerococcum* the initial parental forms differed by the green colour of their foliage — an interesting statement which was not, however, placed in connection with the factorial distribution of the chlorophyll deficiencies on the parental species, as the segregation was not further investigated. Another interesting statement is added: »In specific crosses, closely related, defects of chlorophyll were observed only in hybrids *T. persicum* × *T. dicoccum*, representing distant geographical races. Genetically remote forms are evidently characterized by a deeper difference in chlorophyll factors, probably in the fundamental ones» (l. c., p. 651).

Only one study on chlorophyll deficiencies (in cultivated oats) can be said to have previously touched the problem of geographical chlorophyll factorial distribution and associated questions of polymeric chlorophyll factor phylogeny. This single study is that on the *lutescens* oat form by ÅKERMAN (1922). Two of the three *lutescens* polymeric factors were found in a Danish Probsteier oats (ÅKERMAN and FRÖIER, 1942). The third factor alone was found in several varieties of black-hulled oats from central Sweden. Both groups are ecologically well separated and have a different geographical distribution (MUNDT-PETERSEN, 1937; GRANHALL, 1938).

Reverting to KHINTSHUK's report on *flavescens* plants, he quotes BAUR (1924) regarding the classification of this chlorophyll aberration. According to this, it seems most likely that the *flavescens* plants may be regarded as belonging either to the *chlorina* or the *virescens* groups (DE HAAN's scheme) or *viridis* chief group (GUSTAFSSON, 1940).

(5) SMITH and HARRINGTON (1929), in a cross between the *T. dicoccum* variety Vernal (tetraploid) and the *T. vulgare* variety Marquis, obtained *albinas* in ratios which suggested that one parent carried two recessive polymeric factors, and the other carried one. Perfect ratios were obtained, and the figures for the trimeric segregation were found to be statistically significant. The investigation was not extended so far

as to ascertain which parent carried two polymeric factors, and which of them carried the third. In a review of SMITH and HARRINGTON's results, HUSKINS (1929) judges the conclusions drawn and adds: »From their respective tetraploid and hexaploid nature it seems almost certain that it was *T. dicoccum* that carried the one harmful gene, and *T. vulgare* which was able to carry two such genes without showing their effect» (l. c., p. 30).

(6) In 1932 L. A. SAPEHIN published a report on genes for leaf colour in Emmer wheats. The work has not been accessible to the present author up to time of writing, but a quotation from it is given by PHILP (1935, pp. 291—292): »In crosses between different forms of *T. durum* SAPEHIN found two pairs of factors for albinism and two pairs of factors for yellow seedlings. From crosses between *T. durum* and *T. dicoccum*, however, he concludes that there are three pairs of factors controlling albinism and quotes KHINTSHUK as having obtained a similar result with crosses between *T. Timopheevi* and *T. persicum*.» As for the last-mentioned quotation by SAPEHIN, it should be noted that KHINTSHUK, whose studies of segregation were on quite a small scale, can scarcely be regarded as having demonstrated the existence of trifactorially controlled albinism.

(7) The first known case of undoubted spontaneous mutability of genes for chlorophyll characters in hexaploid wheat is met with in a report by NEATBY (1933). He found a chlorophyll-deficient plant in an F_5 from the cross Garnet $\times$ Double Cross; when crossed with the line from which it arose and with the parental varieties, monohybrid segregation was observed in the F_2. The »yellow» seedling found had its first leaf devoid of chlorophyll except at the tip, where a trace was present. The second leaf emerged pale yellowish-green, but afterwards gradually darkened. At temperatures below + 15 to 16° C. the seedling fails to turn green, thus showing a correspondence with the low-temperature albinism in barley reported by COLLINS (1927; see below, p. 311). The aberrant form — though with some difficulty — may be classified, according to GUSTAFSSON's scheme, as a f. *viridoalbina*. It was unstable: five mutations back to normal were observed in the mutant line. One of these was quite normal and in the next generation gave 84 green, 21 yellow and 1 *albina* plant. As for the *albina* plant, NEATBY (l. c., p. 162) states: »It is probable that it resulted from a mutational change in the gene concerned in the yellow mutation.» Four out of the five mutations recorded were »somatic» and yellowish-striped in sections of varying leaf-width (cf. ÅKERMAN's *luteostriata* oat form, 1933).

(8) NEATBY (1933, p. 159), in reviewing cases of chlorophyll aberration in *Triticum*, reports that, according to information from Dr. T. JOHNSON (unpublished), chlorophyll-deficient seedlings »occur not infrequently in his own cultures of Einkorn (*T. monococcum*)». This statement should be connected with the observations of L. SMITH (1936, pp. 30—31) on »naturally occurring viable mutants» in control plots with untreated *T. monococcum*. Among such spontaneous *viable* mutants in this wheat, SMITH does not, however, mention any chlorophyll aberrant. The present writer has in vain tried to find in the literature any further reports on spontaneous chlorophyll aberrations in *T. monococcum* — or the related diploid *T. aegilopoides*. In view of the greatly restricted cultivation of *T. monococcum* in modern agriculture, such observations must naturally have been extremely scarce. In the writer's own experiments (7 years' growings of limited control plots of the species), no spontaneous chlorophyll aberrant has as yet been found. Only one chlorophyll mutation (f. *tigrina*, Ch. IV) was found during these years in untreated field plots of diploid oats (*Avena strigosa*, *A. brevis*) — in sharp contrast to the results after treatment with weak — medium X-ray dosages in diploid oats and wheat.

(9) In 1937 KIHARA studied chlorophyll defective segregates arising from interspecific hybrids between *Triticum persicum* and *T. Timopheevi*. From free pollination in the highly sterile wheat hybrid *T. persicum* v. *stramineum* × *T. Timopheevi*, 4 F_2 plants were obtained. One of them was fertile. In its progeny normal green, yellow and albinotic plants appeared. The investigation, which was extended to F_5, resulted in the discovery of the existence of one recessive gene *y* for yellow seedlings (*xantha* or *lutescens*), as well as the existence of two recessive genes for *albina*, designated a_1 and a_2. The genes are inherited independently of each other. The formulas for the different chlorophyll characters are:

$$\left.\begin{matrix} A_1\ A_2\ Y \\ A_1\ a_2\ Y \\ a_1\ A_2\ Y \end{matrix}\right\} \text{green} \qquad \left.\begin{matrix} A_1\ A_2\ y \\ A_1\ a_2\ y \\ a_1\ A_2\ y \end{matrix}\right\} \text{yellow} \qquad \left.\begin{matrix} a_1\ a_2\ Y \\ a_1\ a_2\ y \end{matrix}\right\} \textit{albina}$$

The yellow plants may probably be classified as *xantha* types (DE HAAN, l. c.; GUSTAFSSON, l. c.) or else as *lutescens* types. The *xanthas* and *albinas* die off after a few days.

(10) SCHKWARNIKOW (1937) reported some spontaneous chlorophyll mutations procured from aged seeds (7—10 years old) of *Triticum vulgare* var. *ferrugineum sibiricum* and *T. vulgare* var. *albidum* (Line

0604, Saratov). He briefly writes only this: »Es wurden verschiedene Mutationstypen gefunden: Chlorophyllmutationen, dichtständige Ähren, Squareheads, Speltoide, teilweise und völlige Sterilität, Zwerge usw. Die meisten gehörten den letzten 3 Kategorien an» (l. c., p. 193). No further details are given.

4. WHEAT, NON-MENDELIAN INHERITANCE.

As for wheat chlorophyll aberrations exhibiting non-Mendelian inheritance, the recorded cases are extremely scarce, being probably confined to the two following:

(1) ÅKERMAN (1929), in a lecture on lethal factors in wheat and oats, points out certain observations made by I. WÅLSTEDT at the Swedish Seed Association's Östgöta Substation at Linköping (unpublished). WÅLSTEDT, in a field of winter wheat, found a form with yellow-striped leaves. This plant gave rise to seedlings among which a certain percentage were purely yellow and died off quite young. From subsequent investigations on this form, it seems probable that it was inherited in the same manner as the *luteostriata* oat form described by ÅKERMAN (1933).

(2) FRANKEL (1933) reports a case of »mass-occurrence of non-inherited chlorophyll defects in wheat» in 23 pure lines from *T. vulgare*, *T. turgidum* and *T. durum*. No less than 25 plants were found with chlorophyll defective leaves, among them 2 apparently quite albinotic. Others had the 4—5 lowest leaves white-striped. Progenies of 13 striped plants gave rise only to quite normal-green seedlings. The occurrence of these defective plants was not attributed to the preceding severe winter, but rather to plasmatic disturbances.

It should be mentioned that FRANKEL also pointed out the modificability of certain albinisms with temperature. HALLQVIST (1923) describes a chlorophyll-deficient and dwarfed barley strain, developing normal chlorophyll above + 15° C. Between 0° to + 10° C. no chlorophyll was developed. A further clear-cut case is that mentioned by COLLINS (1927) in regard to barley. Here a new barley *albina* type was reported, which appeared phenotypically albinotic only at temperatures below + 7° to + 8° C. When plants of the albino type are grown at temperatures above + 18° to + 19° C., they develop normally. A third case, relating to hexaploid oats, is reported by GASSNER (1915) in regard to land oats from Uruguay (La Plata District). Seedlings of this variety (a winter oat), when brought to sprouting at temperatures of + 1° to + 2° C., show only purely white leaves. In the subsequent development of these white plants a marked retardation takes place. Many of them, having apparently lost the capacity of getting green, after some time die off. Others are arrested in their growth, but successively pass, through greenish, to normal green stages. The leaves seem to get green basally first. Occasionally fully developed upper leaves may still show white tips. Striping may also occur in the ontogenesis of such primarily white-leaved oat plants, grown at low temperature.

TABLE 1. *Spontaneously arisen chlorophyll-aberrant forms of oats and wheat, according to Ch. I.*

Oats			Wheat		
Mendelian inherited:			**Mendelian inherited:**		
albina	NILSSON-EHLE	1913	*albina*	MEISTER	1922
	ZHEGALOV	1920		VAVILOV and JAKUSHKINA	1925
	PHILP	1935		KHINTSHUK	1929
	SMITH, D. C.	1938		SMITH and HARRINGTON	1929
lutescens	ÅKERMAN	1922		SAPEHIN, L. A.	1932
chlorina	ÅKERMAN	1929		NEATBY	1933
albovirescens	ÅKERMAN and FRÖIER	1941		KIHARA	1937
viridoalbina	PHILP	1935	*lutescens*	KIHARA (?)	1937
			chlorina	MEISTER (?)	1922
				KHINTSHUK	1929
			xantha	KHINTSHUK	1929
				SAPEHIN, L. A. (?)	1932
				KIHARA (?)	1937
				HARRINGTON and SMITH	1928
			viridoalbina	NEATBY	1933
			non-described types	SCHKWARNIKOW	1937
				NEATBY	1933
Non-Mendelian inherited:			**Non-Mendelian inherited:**		
luteostriata	CHRISTIE	1921	*luteostriata*	ÅKERMAN (WÅLSTEDT)	1929
	COFFMAN, PARKER and QUISENBERRY	1925	*albostriata*	FRANKEL	1933
	ÅKERMAN	1933			
	ROBB	1933			
	LOVE and CRAIG	1936			
»blotch leaf»	FERDINANDSEN and WINGE	1930			

In contradistinction from plants like *Zea*, *Hordeum*, *Antirrhinum*, *Secale* and many other diploids, the reports on wheat and oat chlorophyll aberrations are thus rare. A concise survey of the above statements in Ch. I is given in Table 1.

II. THE DISTRIBUTION OF CHLORINA FACTORS ON TWENTY-EIGHT HEXAPLOID OAT STRAINS.

1. CROSS COMBINATIONS. NOTES ON THE TECHNIQUE OF CHLORINA OAT CROSSES.

In the report on a spontaneous *chlorina* mutation in *Avena sativa* (ÅKERMAN and FRÖIER, 1941) evidence was given of the monofactorial

constitution of the oat strains Guldregn I (pure line from Milton Probsteier oats), Abeds Nova (pure line from a German Probsteier oats called »Riesen Sommerhafer»; VESTERGAARD, 1928), and 0101 (pure line from Jaune géant à grâppes from »Gelber Flandrischer Hafer») in respect to this chlorophyll aberration. Segerhavre (pure line from Milton Probsteier oats) was shown to be bifactorial. Finally, Klockhavre II (black oat, from central Sweden, most probably originating from a natural cross between Guldregn I and the old Klock I, the latter a pure line from Scotch Black Tartarian oats), and Hede oats (old land variety of Danish origin, grey-hulled), were found to be trifactorial. More than three factors responsible for the *chlorina* character were thus not discovered. Since it must be considered to be of high interest to study further the distribution of *chlorina* factors on some other oat strains of different origin, geographical distribution and agroecotype, the author has so far made complete cross analyses of the following combinations:

Chlorina × *A. sativa* v. *aurea* KÖRN. Pedigree by the present writer from a sample from Turkestan, procured from the Inst. of Applied Botany, Leningrad. According to PETROPAVLOVSKY (1931), in his survey of recently cultivated oat varieties in various parts of the USSR, the v. *aurea* KÖRN. »is of frequent occurrence in the northernmost regions, gradually becoming rarer towards the South. The widest spread is enjoyed by this variety in the Asiatic part of USSR» (l. c., pp. 133—134). — *Chl.* × *Cartier.* Cross-bred white oats from Quebec in Canada. Parents: Alaska × Early Triumph; STANTON, 1936. — *Chl.* × *Fyrishavre.* Pure line from old land oats from Roslagen in the east-coastal parts of Uppland. Black-hulled; NILSSON-EHLE, 1911 b. — *Chl.* × *Gotlandshavre.* White-gray-hulled land oat from Visby, Gotland (»Sommarhavre»); pedigree 1934 by ÅKERMAN. — *Chl.* × *Gul Naesgaardshavre.* Pedigree from German Beseler oats. Selected by VESTERGAARD; cf. MORTENSEN and HANSEN, 1909. Yellow hulls, kernels of Probsteier type. — *Chl.* × *Ligowo II.* White oats; pedigree selection from VILMORIN's Ligowo oats. The latter, according to some writers' opinion, originates from the Baltic provinces; cf. MORTENSEN and HANSEN, l. c. Other authors, e. g., ZADE (1918), suppose the variety to originate from the Pyreneans. The morphological characters of Ligowo II, however, speak distinctly in favour of the Baltic origin. — *Chl.* × *v. Lochow's Flämminggold.* From the cross v. Lochow's Gelbhafer × »ungezüchteter Oberschlesischer Weisshafer». The former »eine Märkische Landsorte»; Sortenregister des Reichsnährstandes, 1936. — *Chl.* × *Mansholt's Binder.* Dutch, white- or white-yellow-hulled oats. A line from the German Carsten's Oat III, derived by pedigree selection from Carsten II. — *Chl.* × *Mesdag.* »Avoine précoce de Mesdag», pedigree selection by VILMORIN and ANDRIEUX from Dutch oats (DENAIFFE et SIRODOT, 1901). The primary origin of the variety is, however, traced back to the Baltic provinces (ÅKERMAN, NILSSON-EHLE and HOLMGREN, 1921), perhaps to Finland. Marketed 1894. Black-hulled, extremely early. — *Chl.* × *Orion II.* North-Swedish early black oats, cross-bred from Ligowo II × 0668, the latter a pure line from early north-Norwegian land oats from Merø, Nordlands fylke; ÅKERMAN, 1927. — *Chl.* × *Stormogul I.* Central-Swedish black oats, pure line from Scotch Black Tartarian oats; N. HJ. NILSSON, 1899. — *Chl.* × *Thorshavre.* Pedigree by CHRISTIE from Norwegian Hedemarkshavre from Vindjur, Ringsaker. A typical representative of Scandinavian white oats; CHRISTIE, 1917. — *Chl.* × *0660.* Pure line from early north-Finnish black land oats. Cf. ATTERBERG, 1889; NILSSON-EHLE, 1921. — *Chl.* × *0668.* Pure line from early north-Norwegian black land oats. Cf. above Orion II. — *Chl.* × *0691, Brun Ölandshavre.* Pedigree from old

land oat from the island of Öland in the Baltic Hulls grey-brown, early ripening; N. HJ. NILSSON, 1898.

Furthermore, following cross analyses though not yet completely finished, may be recorded, as the results permit valuable conclusions to be drawn in connection with the problem of *chlorina* factor distribution:

Chlorina × *A. byzantina* (*A. sterilis* L. ssp. *byzantina* [KOCH] THELLUNG). Procured from Algier by seed exchange with the aid of the late Dr. FREISLEBEN, Halle (Saale), Germany. — *Chl.* × *A. sativa* v. *inermis* KÖRN. (*A. sativa* ssp. E. *A. nuda* II. a. *chinensis* ASCHERSON and GRAEBNER, 1899). Naked oats, originating from China. Sample procured from Kai-foeng, province of Ho-Nan, through Dr. FREISLEBEN, Halle. — *Chl.* × *A. sterilis* ssp. *macrocarpa* (MÖNCH) BRIQUET (*A. sterilis* L. *s. str.*, *A. sterilis typica* REGEL, etc.). A strain originating from the town of Ronda in Granada. Seeds procured from Kaiser Wilhelm-Institut für Züchtungsforschung, Müncheberg. — *Chl.* × *Dalslandshavre* 29/100. Pure line from a grey- and white-hulled land oat population from Lommelanda, Göteborgs och Bohus län. »Primary group V 15, biotype No. 29 d»; GRANHALL, 1938, p. 74, p. 97. — *Chl.* × *Förädlad Dalahavre*. Pure line from north-Swedish white oats. Cf. NILSSON-EHLE, 1907 b. — *Chl.* × *Nidarhavre*. A line from north-Norwegian Trønderhavre, selected by GLAERUM. White-hulled, extremely early. Cf. VIK, 1932. — *Chl.* × *Smålandshavre* (*Spetskornhavre*) 29/37. Pure, white-hulled line from a land oat population from Näshult, Jönköpings län. »Primary group V 2, biotype No. 5; GRANHALL, 1938, p. 74, p. 91.

The crosses between the *chlorina* triple recessive (from Guldregn I) were carried out in 1939—1943. Since the *chlorina* recessive shows a somewhat retarded development (cf. ÅKERMAN and FRÖIER, 1941), certain difficulties were encountered in the crossing work. Thus, there were several cross combinations which could not be effected in spite of repeated crossing for two or three years. Even by earlier sowing of the recessive form in the greenhouse the difficulties could not be completely overcome, owing to the restricted seed-setting under such conditions. Cultures in open greenhouses or open frames were found to give best results. At Svalöf the oat emasculations are made during the whole day (with the exception of the hottest hours, about noon, in dry and sunny periods). 15—30 flowers per panicle are emasculated. Only one flower per spikelet is used, the others are removed. The panicle is kept protected by a parchment bag for three days. Pollination is then carried out in the morning or the afternoon hours (7—9 a. m., 3—5 p. m.). At Svalöf the pollinations from the morning hours have been found to give best seed-setting, this in deviation from reports on oat crossing technique from later years by COFFMAN (1937) and MISONOO (1936). COFFMAN, for instance, found the most favourable time for crossing oats to be between 2 and 5 o'clock in the afternoon, at which time blooming culminates. Some idea of the seed-setting in the *chlorina* crosses at Svalöf is given by the following tabulation, comprising medium percentages of seed-setting 1939 up to 1943 in crosses *chlorina* × all tried strains belonging to *A. sativa s. str.* Seed-setting percentage = number of F_1 kernels procured/number of emasculated and pollinated flowers. By way of comparison, the medium percentages of all oat crossings carried out for practical breeding purposes in the Wheat and Oats Dept. in the same years have been included:

	1939	1940	1941	1942	1943
Chlorina crosses, %	13,7	6,7	2,4	6,7	2,8
Practical breeding crosses, %	12,7	40,4	6,3	3,6	9,6

Thus, the above percentages indicate that at least in certain years the *chlorina* crosses have yielded a rather limited amount of F_1 kernels. On an average for the period 1939—1943, they show a seed-setting percentage of 7,1 % (2.630 pollinated flowers). In the practical breeding oat crosses the corresponding percentage for the

same period is 12,2 % (11.002 pollinated flowers). COFFMAN (l. c., p. 303) from 12 years' crossing work reports an average percentage of slightly less than 10 %; this resulted from pollination of more than 5.000 flowers.

As a rule the triple *chlorina* recessive was chosen as ♀ parent, the F_1 control thus being very simple. In some combinations, however, the recessive had to be used as ♂.

2. GENETICAL RESULTS.

Chlorina × *A. sativa* v. *aurea* KÖRNICKE. — From cross No. 319 in 1942 three F_1 kernels were obtained. Grown in 1943 as No. 961 r 7, only two F_1 plants developed to maturity. In 1944 the two resulting F_2 progenies, 967 r 1 and r 2, segregated: the former in 16 *chlorina*: 47 green, the latter in 5 *chlorina*: 18 green. In short, 21 : 65 (for a 3 : 1 segregation the expected ratio was 21,5 : 64,5). The variety thus contains one homologous, polymeric *Chlor* factor.

Chlorina × *Cartier*. — From cross No. 127 and No. 128 + No. 129 in 1939 four and three F_1 kernels respectively were obtained, grown in 1940 as No. 950 r 31_{1-4} and r 33_{1-3}. In 1941 the seven resulting F_2 progenies segregated as shown in the table below.

Field number of F_2	Number of seeds sown	Number of seedlings	Found: *chlorina*/ green	Expected: *chlorina*/ green	P-values for a 3 : 1 ratio
952 r 16	132	113	27 : 86	28,3 : 84,7	$0{,}80 > P > 0{,}70$
17	250	226	48 : 178	56,5 : 169,5	$0{,}20 > P > 0{,}10$
18	165	131	22 : 109	32,8 : 98,2	$0{,}05 > P > 0{,}02$
19	174	146	24 : 122	36,5 : 109,5	$0{,}02 > P > 0{,}01$
20	447	364	86 : 278	91,0 : 273,0	$0{,}70 > P > 0{,}50$
21	456	373	69 : 304	93,3 : 279,7	$0{,}01 > P > 0{,}001$
22	31	27	6 : 21	6,8 : 20,2	$0{,}80 > P > 0{,}70$
	1.655	1.380	282 : 1.098	345 : 1.035	$P < 0{,}001$***

The variety unquestionably contains one *chlor* factor. The significantly deviating F_2 progenies 21, 19 and 18 all show a marked deficit in recessives, owing to the extremely dry spring of 1941. In progeny 21 further severe attacks of the frit fly were observed, in the first place delaying and destroying the weaker *chlorina* recessives.

Chlorina × *Fyrishavre*. — From cross No. 343 in 1942 two F_1 kernels were obtained, grown in 1943 as No. 961 r 10_{1-2}. In 1944 the two resulting F_2 progenies segregated, No. 967_6 in 4 *chlorina* : 148 green (theoretically for a 63 : 1 ratio we have 2,4 : 149,6), and No. 967_7 in 4 *chlorina* : 59 green (theoretically for a 15 : 1 ratio we have 3,94 : 59,06). Only an F_3 analysis can differentiate between the two possibilities, F_2 being so limited.

The F_3 growings were carried out in wet sand in porcelain dishes in full light in the greenhouse or in the assorting-rooms of the Seed Association at a temperature

of + 18° C. The dishes were watered daily, and the desired optimal conditions for rapid germination in full light were attained. The number of kernels sown in each dish was always counted. In this manner the germination percentage could be determined and possible deficits in the numbers of recessives be accounted for. In fact, however, no such deficits manifested themselves in these large F_3 growings, and the germination percentages of the segregating progenies never significantly deviated from those of fully normal green, non-segregating ones. Both groups of germination percentage numbers were high, about 92—98 %.

100 normal-green plants from the F_2 progeny 1944—967$_6$ were thus grown in porcelain dishes in wet sand. The following results were obtained:

Dish number	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Most probable ratio of F_3 progeny
1	97	95	94	1	63 : 1
2	84	76	72	4	15 : 1
3	102	98	92	6	15 : 1
4	45	43	30	13	3 : 1
5	54	52	all	—	—
6	105	101	98	3	63 or 15 : 1 ?
7	141	135	all	—	—
8	69	68	67	1	63 : 1
9	68	65	all	—	—
10	127	120	all	—	—
11	75	72	all	—	—
12	69	67	62	5	15 : 1
13	67	65	all	—	—
14	97	93	all	—	—
15	37	32	24	8	3 : 1
16	36	35	all	—	—
17	25	24	all	—	—
18	52	50	all	—	—
19	10	10	all	—	—
20	25	23	19	4	3 : 1
21	93	90	89	1	63 : 1
22	102	100	all	—	—
23	51	49	41	8	3 : 1
24	39	37	all	—	—
25	52	51	36	15	3 : 1
26	18	18	11	7	3 : 1
27	48	45	36	9	3 : 1
28	10	10	all	—	—
29	91	86	85	1	63 : 1
30	37	36	all	—	—
31	109	103	98	5	15 : 1
32	25	22	20	2	15 : 1 ?
33	56	54	all	—	—
34	40	38	all	—	—
35	64	63	all	—	—
36	33	33	30	3	15 : 1 ?
37	58	58	57	1	63 : 1
38	64	58	54	4	15 : 1
39	9	9	all	—	—
40	47	45	37	8	3 : 1
41	234	230	all	—	—
42	204	200	all	—	—

Dish number	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Most probable ratio of F_3 progeny
43	231	215	213	2	63 : 1
44	66	65	all	—	—
45	103	98	all	—	—
46	129	125	all	—	—
47	148	139	105	34	3 : 1
48	51	48	40	8	3 : 1
49	153	150	all	—	—
50	60	60	all	—	—
51	16	9	all	—	—
52	135	130	all	—	—
53	132	125	all	—	—
54	110	104	103	1	63 : 1
55	35	33	all	—	—
56	96	94	all	—	—
57	108	105	all	—	—
58	47	42	39	3	15 : 1
59	31	31	all	—	—
60	81	78	74	4	15 : 1
61	81	80	all	—	—
62	162	153	all	—	—
63	30	28	all	—	—
64	69	65	all	—	—
65	207	203	all	—	—
66	21	21	all	—	—
67	81	73	72	1	63 : 1
68	6	6	all	—	—
69	217	209	198	11	15 : 1
70	123	120	all	—	—
71	52	50	47	3	15 : 1
72	61	58	all	—	—
73	15	12	all	—	—
74	63	60	50	10	3 : 1
75	75	73	69	4	15 : 1
76	65	61	54	7	15 or 3 : 1 ?
77	19	18	all	—	—
78	156	149	all	—	—
79	210	200	all	—	—
80	249	240	227	13	15 : 1
81	75	70	all	—	—
82	123	120	all	—	—
83	45	43	41	2	15 : 1
84	98	95	all	—	—
85	112	105	100	5	15 : 1
86	108	100	96	4	15 : 1
87	51	49	46	3	15 : 1
88	27	23	18	5	3 : 1
89	53	50	38	12	3 : 1
90	61	59	all	—	—
91	42	40	all	—	—
92	37	35	all	—	—
93	43	40	all	—	—
94	37	34	all	—	—
95	35	32	all	—	—
96	67	64	all	—	—
97	96	92	all	—	—
98	56	55	50	5	15 : 1
99	102	98	all	—	—
100	33	30	28	2	15 : 1

Not reckoning the progenies 6, 32, 36 and 76, the most probable mode of segregation of which cannot be ascertained, we thus have 96 F_3 progenies in the following distribution : 58 constant green : 8 trimerically segregating : 17 dimerically and 13 monomerically segregating. The expected values would be $56{,}_{38} : 12{,}_{19} : 18{,}_{29} : 9{,}_{14}$. χ^2 amounts to $3{,}_{208}$, and we have $0{,}_{50} > P > 0{,}_{30}$. Thus, the Fyris oats must possess all three *Chlor* factors hitherto found. On the basis of the F_3 distribution we are, however, able to carry the statistical analysis one step further. Although there are no indications from the small F_2 of the existence of a *tetrameric* segregation, the F_3 progenies are on an average much too small to reveal any truly 255 : 1-segregating family. If now a real tetrameric segregation displayed itself among recorded 96 F_3 families, we should then theoretically expect

$65{,}_{88}$ constant green,
$6{,}_{02}$ 255 : 1-segregating
$12{,}_{05}$ 63 : 1-segregating,
$9{,}_{04}$ 15 : 1-segregating, and, finally,
$3{,}_{01}$ 3 : 1-segregating progenies,

the 255 : 1-segregating ones of course being counted as »constant green». We should then have reckoned $71{,}_{90}$ »green» families in F_3. Now in a correct use of the χ^2 method, if the expected frequency in a certain class is less than five, the class has to be merged in the nearest one (BONNIER and TEDIN, 1940). If we thus count the di- and monomerically segregating progenies together as a single class, we get the three corresponding classes:

Found:	*58* (green + supposed 255 : 1)	*8* (63 : 1)	*30* (15 : 1 + 3 : 1)
Expected:	*71,90*	*12,05*	*12,05* (for 255 : 1)

χ^2 amounts to $30{,}_{787}$; we have $P < 0{,}_{001}$ ***. *Thus, beyond reasonable doubt the Fyris oats does not contain more than three Chlor factors.*

Chlorina × *Gotlandshavre.* — From cross No. 329 in 1942 only one F_1 kernel was procured, grown in 1943 as No. 961 r 9. In 1944 the single resulting F_2, No. 967 r 5, segregated in 5 *chlorina* : 20 green, thus indicating a monomeric segregation. Of the 20 green plants, 18 were grown in sand dishes as an F_3 (2 plants happened to be mixed in the

thrashing and thus had to be discarded); the segregation of the F_3 families was recorded as follows:

Dish number	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Most probable ratio of F_3 progeny
1	30	28	23	5	3 : 1
2	42	42	35	7	3 : 1
3	46	40	32	8	3 : 1
4	207	200	all	—	—
5	198	182	142	40	3 : 1
6	152	145	107	38	3 : 1
7	103	101	all	—	—
8	108	105	75	30	3 : 1
9	6	6	all	—	—
10	45	43	33	10	3 : 1
11	153	145	107	38	3 : 1
12	144	135	105	30	3 : 1
13	204	195	all	—	—
14	195	190	140	50	3 : 1
15	126	120	85	35	3 : 1
16	142	139	all	—	—
17	300	285	all	—	—
18	162	153	111	42	3 : 1

Among the 18 F_3 progenies, 12 segregated according to 3 : 1 and 6 were non-segregating, which quite corresponds with the theoretical expectation. Thus, Gotlandshavre unquestionably carries only one *Chlor* factor.

Chlorina × *Gul Naesgaardshavre.* — From cross No. 357 and No. 359 in 1941 one F_1 kernel each was obtained, grown in 1942 as No. 946 r 21 and r 22. In 1943 the two resulting F_2 progenies 962 r 26 and r 27 gave the following segregation:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives
r 26	30	24	17	7
r 27	147	141	112	29
	177	165	129	36

The expected values for a 3 : 1-segregation would be $123{,}7 : 41{,}3$. χ^2 amounts to $0{,}907$ with $0{,}50 > P > 0{,}30$. Thus, Gul Naesgaardshavre must carry only one *Chlor* factor.

Chlorina × *Ligowo II.* — From cross Nos. 120, 122 and 123 in 1939 one F_1 kernel each was obtained, grown in 1940 as No. 950 r 23, 24 and 25. In 1941 the three resulting F_2 progenies 952 r 13, 14 and 15 gave the following segregation:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives
r 13	241	192	142	50
r 14	369	317	240	77
r 15	2	1	—	1
	612	510	382	128

The small F_2 progeny r 15 originates from a fully green F_1 plant, i. e. from a real cross, and must thus be reckoned together with the other progenies. The expected segregation numbers are 381,5 : 127,5, which are in the closest correspondence with the numbers found, 382 : 128. Ligowo II oats thus carries only one *Chlor* factor.

Chlorina × *v. Lochow's Fläminggold.* — From cross Nos. 140, 141 and 144 in 1939, respectively, one, two and one real F_1 plants were procured, grown in 1940 as No. 950 r 36, r 38_{1-2} and r 42_2. In 1941 the four resulting F_2 progenies 952 r 24, 25, 26 and 28 gave the following segregation:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Expected green : *chlorina*	P-values for a 3 : 1 ratio
r 24	405	350	291	59	262,5 : 87,5	$P < 0{,}001$
r 25	585	486	406	80	364,5 : 121,5	$P < 0{,}001$
r 26	897	707	575	132	530,2 : 176,8	$P < 0{,}001$
r 28	846	709	562	147	531,7 : 177,3	$0{,}01 > P > 0{,}001$
	2.733	2.252	1.834	418	1.689,0 : 563,0	$P < 0{,}001$

All four F_2 progenies thus significantly differ from the expected monomeric ratios. The number of recessives in each progeny shows a marked deficit. This result is, however, easily accounted for, since the extremely dry spring of 1941 together with attacks of the frit fly (cf. p. 315, cross *chlorina* × Cartier, progenies 21, 19 and 18) must have caused this deficit. Only 82 % of the seeds sown developed seedlings, and it was observed that the *chlorina* recessives this year were greatly delayed in their development, causing many difficulties in recognizing the most retarded ones as *chlorinas*. For this latter reason also a certain number of ill-developed seedlings must have been classified as normal green. As for the number of *Chlor* factors in v. Lochow's Fläminggold, *there cannot be more than one*, in view of the segregations reported above.

Chlorina × *Mansholt's Binder.* — From cross No. 147 and No. 148 in 1939 one F_1 kernel each was obtained, grown in 1940 as No. 950 r 45 and 46. In 1941 the two resulting F_2 progenies 952 r 29 and r 30 gave the following segregation:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Expected green: *chlorina*	P-values for a 3 : 1 ratio
r 29	606	572	474	98	429,0 : 143,0	$P < 0{,}001$
r 30	540	473	391	82	354,7 : 118,3	$P < 0{,}001$
	1.146	1.045	865	180	783,7 : 261,3	$P < 0{,}001$

Just as in regard to the above-mentioned cross *chlorina* × v. Lochow's Flämminggold, the F_2 progenies significantly differ from the supposed monomeric ratios of segregation. The marked deficit of recessives is accounted for by their greatly retarded development and the difficulty of classifying certain badly developed seedlings. In general, the year 1941 was not very opportune for determining F_2 segregations of chlorophyll aberrant forms.

From the results procured it should, however, be evident that Mansholt's Binder oats carries only one *Chlor* factor.

Chlorina × *Mesdag.* — Crosses Nos. 196, 199, 200 and (the reciprocal) 201, 202, 203, 205 in 1940 yielded in 1942 no less than 10 unquestionably monomerically segregating F_2 progenies, recorded below:

F_2 progeny number	Found: green	*chlorina*	F_2 progeny number	Found: green	*chlorina*
941 r 40	127	32	941 r 46	60	17
41	171	59	47	107	41
43	304	73	48	266	68
44	211	62	49	205	54
45	107	25	50	291	82

In total we get 1849 green : 513 *chlorina*; expected 1771,5 : 590,5; $P < 0{,}001$. The obvious deficit of recessives must, however, be accounted for by their greatly retarded development.

Mesdag oats is thus unifactorial in respect to *chlorina* factors.

Chlorina × *Orion II.* — From cross No. 133 in 1939 one F_1 kernel was obtained, which gave rise to a single F_2 progeny: 1941 — 952 r 23, segregating in 334 green : 20 *chlorina.* For a 15 : 1 ratio we should have expected 331,9 : 22,1; $\chi^2 = 0{,}213$; $0{,}70 > P > 0{,}50$, i. e. a very good correspondence. 75 of the 334 green plants were thrashed and grown as an F_3 in sand dishes in the laboratory — with a view to further verification — and thus 44 green, 15 dimerically and 16 monomerically segregating families were found. The expected distribution would be 35 : 20 : 20; $\chi^2 = 4{,}364$, and we find $0{,}20 > P > 0{,}10$. The distribution does not accord with any supposed trimeric segregation: in such case the expected distribution would be 53,6 : 14,3 : 7,1 — the hypothetical non-revealed 63 : 1-segregating families being then reckoned as green ones; χ^2 then amounts to 12,910, and we have $0{,}01 > P > 0{,}001$. Thus, the distribution, beyond reasonable doubt, accords with the 15 : 1 ratio; Orion II therefore has two *Chlor* factors.

Chlorina × *Stormogul I.* — Cross No. 416 from 1938 gave rise to the two F_1 plants 1939—958—5 a and 5 b; the latter gave the F_2 progenies 1940—951 r 13 and r 14, segregating as follows:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Found: green/ *chlorina*	Expected: green/ *chlorina*	P-values for a 15 : 1 ratio
r 13	207	176	169 : 7	165 : 11	$0{,}30 > P > 0{,}20$
r 14	371	201	194 : 7	188,4 : 12,6	$0{,}20 > P > 0{,}10$
	578	377	363 : 14	353,4 : 23.6	$0{,}05 > P > 0{,}02$

The obvious deficit of the recessives can be accounted for by the low germination percentage, merely 65,2 %. The kernels from the F_1 plants were unfortunately not well developed. From the F_2 progeny r 13 20 pure green plants were thrashed and grown in sand dishes as an F_3. The results are tabulated below:

Progeny number of F_3	Number of seeds sown	Number germinated seeds	Per cent germinated seeds	Found: green/ *chlorina*	Expected: green/ *chlorina*	P-values	Mode of segregation
1	186	172	92,5	all green	—	—	—
2	179	162	90,5	» »	—	—	—
3	221	207	93,7	189 : 18	194,1 : 12,9	$0{,}20 > P > 0{,}10$	15 : 1
4	359	325	90,5	all green	—	—	—
5	61	56	91,8	52 : 4	52,5 : 3,5	$0{,}80 > P > 0{,}70$	15 : 1
6	330	287	87,0	226 : 61	215,2 : 71,8	$0{,}20 > P > 0{,}10$	3 : 1
7	66	64	97,0	all green	—	—	—
8	82	80	97,6	» »	—	—	—
9	267	260	97,4	» »	—	—	—
10	84	81	96,4	60 : 21	60,7 : 20,3	$0{,}90 > P > 0{,}80$	3 : 1
11	97	92	94,8	all green	—	—	—
12	247	236	95,5	» »	—	—	—
13	304	285	93,8	» »	—	—	—
14	103	90	87,4	65 : 25	67,5 : 22,5	$0{,}70 > P > 0{,}50$	3 : 1
15	105	100	95,2	all green	—	—	—
16	121	111	91,7	» »	—	—	—
17	110	98	89,1	» »	—	—	—
18	60	54	90,0	» »	—	—	—
19	186	180	96,8	170 : 10	168,7 : 11,3	$0{,}70 > P > 0{,}50$	15 : 1
20	81	76	93,8	all green	—	—	—

Thus green, di- and monomerically segregating families are found in the ratio 14 : 3 : 3 (expected values are 9,4 : 5,3 : 5,3); $\chi^2 = 4{,}247$, and we find $0{,}20 > P > 0{,}10$, i. e. a satisfactory correspondence with the estimate. As a curiosity, it must be pointed out, however, that if a trimeric distribution of the 20 F_3 families is assumed, an excellent correspondence with this hypothesis will be found. To the found numbers

14 : 3 : 3 we would then have the corresponding expected numbers $(11{,}8 + 2{,}5) : 3{,}8 : 1{,}9$, i. e. $14{,}3 : 3{,}8 : 1{,}9$. If the two last classes are taken together, we get:

Found	14	green	: 6	di-	and monomerically	segregating
Expected	14,3	»	: 5,7	»	»	»

$\chi^2 = 0{,}022$; $0{,}90 > P > 0{,}80$. This astonishingly good correspondence must, however, be the result of mere chance. The two F_2 progenies r 13 and r 14 (p. 322) can by no means be supposed to be trimerically segregating, since the writer's experience from the delayed development of the *chlorina* recessives in the open field unquestionably justifies the assumption that a better quality of the kernels from the F_1 plants would have yielded still some more recessives in these two F_2 progenies. In these F_2 progenies the χ^2 values for a supposed trimeric segregation are 6,402 and 4,983 with the P-values $0{,}02 > P > 0{,}01$ and $0{,}05 > P > 0{,}02$. As compared with the P-values for a dimeric segregation, at any rate the first value ($0{,}02 > P > 0{,}01$ for r 13) cannot fit a trimeric segregation in this progeny. Thus, Stormogul I must contain two *Chlor* factors.

Chlorina × *Thorshavre*. — Cross Nos. 348 and 350 from 1941 yielded good F_1 harvest 1942, and in 1943 11 F_2 progenies grew in the field and segregated as tabulated below:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives
962 r 15	171	153	147	6
16	233	216	214	2
17	192	151	151	—
18	121	113	107	6
19	276	242	239	3
20	219	175	175	—
21	268	255	249	6
22	71	55	55	—
23	157	142	138	4
24	66	60	58	2
25	106	80	80	—
	1.880	1.642	1.613	29

Now, the F_2 progenies r 17, 20, 22 and 25 are unquestionably true crosses. Hence they must be reckoned together with the segregating families. Obviously, we are confronted here with a trimeric segregation. For 63 : 1 the expected numbers are $1616{,}3 : 25{,}7$; $\chi^2 = 0{,}430$ and $0{,}70 > P > 0{,}50$, i. e. a very good correspondence. For a supposed dimeric segregation we get $\chi^2 = 56{,}316$ and $P < 0{,}001$. Finally, for a supposed

tetrameric segregation (expected numbers 1635,6 : 6,4) we get $\chi^2 = 80{,}119$ and $P < 0{,}001$. In this case it was thus possible — without the aid of a large F_3 generation — to prove beyond reasonable doubt that Thorshavre contains *three Chlor* factors.

Chlorina × *0660.* — Cross No. 313 from 1941 yielded a single F_1 kernel which gave rise to the F_2 progeny 1943—962 r 14. From 198 seeds sown 142 green and 43 *chlorina* plants were recorded (we should have expected for a 3 : 1 ratio 138,7 : 46,3). For a monomeric ratio we have $\chi^2 = 0{,}314$ and $0{,}70 > P > 0{,}50$. A supposed dimeric ratio would give the expected values 173,4 : 11,6; $\chi^2 = 90{,}683$; $P < 0{,}001$. The variety 0660 contains *only one Chlor* factor.

Chlorina × *0668.* — Cross No. 325 from 1942 yielded in 1944 the two F_2 progenies 967 r 3 and r 4 with 55 and 17 sown kernels respectively. Neither of the two progenies segregated. Since, however, a di- or trimeric segregation must have been latent here (both progenies were unquestionably true crosses), 44 plants from r 3 were thrashed and grown as an F_3 in sand dishes. The results are tabulated below:

Dish number	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Most probable ratio of F_3 progeny
1	79	74	all	—	—
2	45	40	all	—	—
3	29	24	19	5	3 : 1
4	47	46	all	—	—
5	71	67	47	20	3 : 1
6	38	36	all	—	—
7	66	62	all	—	—
8	85	82	78	4	15 : 1
9	21	20	all	—	—
10	16	15	all	—	—
11	69	65	62	3	15 : 1
12	13	12	all	—	—
13	5	5	all	—	—
14	35	32	all	—	—
15	117	113	all	—	—
16	88	85	65	20	3 : 1
17	6	6	all	—	—
18	25	24	all	—	—
19	3	3	all	—	—
20	29	28	23	5	3 : 1
21	97	86	80	6	15 : 1
22	9	8	all	—	—
23	130	125	all	—	—
24	91	88	all	—	—
25	30	29	21	8	3 : 1
26	177	149	140	9	15 : 1
27	120	115	all	—	—
28	70	73	all	—	—
29	45	44	all	—	—
30	102	95	75	20	3 : 1

Dish number	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Most probable ratio of F_3 progeny
31	86	80	76	4	15 : 1
32	58	57	53	4	15 : 1
33	160	150	all	—	—
34	290	252	137	15	15 : 1
35	103	95	all	—	—
36	88	82	65	17	3 : 1
37	90	88	84	4	15 : 1
38	75	71	56	15	3 : 1
39	36	30	22	8	3 : 1
40	102	95	all	—	—
41	58	53	40	13	3 : 1
42	110	103	73	30	3 : 1
43	92	92	88	4	15 : 1
44	72	71	all	—	—

Thus, the proportions of green, di- and monomerically segregating F_2 progenies found were 24 : 9 : 11. We should have expected (for a 15 : 1 distribution) $20{,}6 : 11{,}7 : 11{,}7$; $\chi^2 = 1{,}226$ and $0{,}70 > P > 0{,}50$, i. e. a very good correspondence. For a supposed trimeric segregation the expected proportions would be $31{,}4 : 8{,}4 : 4{,}2$ (the hypothetic 63 : 1-segregating families reckoned as green ones); $\chi^2 = 12{,}797$ and $0{,}01 > P > 0{,}001$. Thus, beyond reasonable doubt, the oat strain 0668 carries *two Chlor* factors.

Chlorina × *0691, Brun Ölandshavre.* — Cross Nos. 345 and 346 from 1942 yielded 4, respectively 5 F_1 kernels, resulting in 9 F_2 progenies, 967 r 8 — r 16 in 1944. The F_2 segregations tabulated as follows:

Field number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives
967 r 8	119	103	100	3
9	5	5	5	—
10	10	8	8	—
11	91	76	72	4
12	56	46	45	1
13	94	73	70	3
14	125	107	103	4
15	50	42	40	2
16	10	9	9	—
	560	469	452	17

The progenies 9, 10 and 16 are true crosses. The expected segregation for a 63 : 1 ratio would be $461{,}7 : 7{,}3$; $\chi^2 = 13{,}093$ and $P < 0{,}001$. For a 15 : 1 ratio it would be $439{,}7 : 29{,}3$; $\chi^2 = 5{,}508$ and $0{,}02 > P > 0{,}01$. The growing of an F_3 is thus indispensable. From 967 r 8 65 plants were thrashed and grown in sand dishes; the results on pp. 326—327.

Thus, green, tri-, di- and monomerically segregating F_3 progenies

Dish number	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Most probable ratio of F_3 progeny
1	118	112	all	—	—
2	58	55	all	—	—
3	119	114	all	—	—
4	66	61	48	13	3 : 1
5	206	200	all	—	—
6	144	136	130	6	15 : 1
7	32	30	all	—	—
8	139	130	all	—	—
9	40	39	all	—	—
10	121	116	109	7	15 : 1
11	39	38	all	—	—
12	32	30	all	—	—
13	119	112	all	—	—
14	44	39	35	4	15 : 1
15	141	138	all	—	—
16	34	32	all	—	—
17	104	102	all	—	—
18	116	110	all	—	—
19	143	137	132	5	15 : 1
20	129	123	all	—	—
21	15	14	all	—	—
22	47	43	41	2	15 : 1
23	110	105	100	5	15 : 1
24	106	100	92	8	15 : 1
25	146	140	all	—	—
26	46	45	all	—	—
27	105	100	all	—	—
28	40	35	33	2	15 : 1
29	116	109	all	—	—
30	32	30	28	2	15 : 1
31	29	26	18	8	3 : 1
32	10	10	all	—	—
33	57	54	all	—	—
34	151	143	all	—	—
35	127	120	114	6	15 : 1
36	76	74	all	—	—
37	54	52	all	—	—
38	102	98	97	1	63 : 1
39	83	80	all	—	—
40	51	49	all	—	—
41	37	36	all	—	—
42	30	28	all	—	—
43	144	139	all	—	—
44	145	140	all	—	—
45	34	32	29	3	15 : 1
46	54	52	42	10	3 : 1
47	87	84	all	—	—
48	102	95	all	—	—
49	87	85	all	—	—
50	195	182	141	41	3 : 1
51	54	52	all	—	—
52	112	108	102	6	15 : 1
53	158	148	all	—	—
54	178	172	170	2	63 : 1
55	99	93	79	14	3 : 1
56	112	108	all	—	—
57	162	154	152	2	63 : 1

Dish number	Kernels sown	Seedlings procured, altogether	Normal green ones	*Chlorina* recessives	Most probable ratio of F_3 progeny
58	274	260	all	—	—
59	57	54	all	—	—
60	82	80	all	—	—
61	105	102	all	—	—
62	164	160	all	—	—
63	98	92	87	5	15 : 1
64	140	138	136	2	63 : 1
65	75	70	66	3	15 : 1

were found in the ratio 42 : 4 : 14 : 5. We should have expected (for a trimeric segregation) $38{,}2 : 8{,}2 : 12{,}4 : 6{,}2$; $\chi^2 = 2{,}968$ and $0{,}50 > P > 0{,}30$, i. e. a good correspondence. According to a 255 : 1-segregation hypothesis, we should get the expected classes $48{,}7 : 8{,}1 : 8{,}2$ to compare with the found ones 42 : 4 : 19 (the first and the third classes being procured by reckoning together primary classes). $\chi^2 =$ amounts to $17{,}221$ with $P < 0{,}001$. Thus, beyond reasonable doubt 0691, Brun Ölandshavre, carries *three Chlor* factors.

The following seven cross analyses have not yet been advanced up to the establishment of a certain number of *chlor* factors as the only possible one.

Chlorina × *A. byzantina* (KOCH) THELLUNG. — Cross No. 300 from 1941 yielded a single F_2 progeny 1943; 12 sown kernels gave 7 non-segregating green seedlings. In 1944 the 7 plants gave 7 F_3 progenies, four constant green, and three, segregating *chlorina* : green in the ratios 3 : 10, 1 : 7 and 1 : 49. The last F_3 progeny was harvested and the green plants grown in sand dishes as F_4 progenies, in order at any rate to determine whether a dimeric segregation was displayed in the primary F_2 progeny. 48 F_4 progenies were procured (one did not germinate), among which were 27 green, 5 *trimerically* segregating (numbers found: 2 : 83, 1 : 94, 2 : 108, 1 : 51 and 1 : 66), 11 dimerically segregating and 5 monomerically segregating. The theoretical distribution would have been $28{,}2 : 6{,}1 : 9{,}1 : 4{,}6$ for a trimeric ratio. If the last two classes are reckoned together, we get $\chi^2 = 1{,}095$ and $0{,}70 > P > 0{,}50$, i. e. a good correspondence. On the basis of the limited F_2 and F_3, we can thus only conclude that the *A. byzantina* strain used must carry *at least three Chlor* factors.

Chlorina × *A. chinensis* ASCH. et GRAEBN. (*inermis* KÖRNICKE). — Cross Nos. 341 and 494 from 1941 yielded two F_2 progenies in 1943, field numbers 1943—962 r 4 and r 5, both segregating. The former segregated into 1 *chlorina* : 36 green (46 kernels sown), the latter into

1 *chlorina* : 44 green (48 kernels sown). From the former progeny 34 green plants were grown in the field as F_3. Unfortunately, the seed-setting of the F_2 plants in this cross was rather poor; hence the F_3 segregations could not satisfactorily be interpreted, nor the distribution of the various segregating progenies calculated. The 34 F_3 progenies consisted of 26 non-segregating and 8 segregating ones, the latter showing the ratios 1 : 16, 1 : 27, 1 : 29, 3 : 66, 1 : 38, 3 : 22 and 3 : 27 *chlorina* : green. From the fourth of the last-mentioned progenies (segregating 3 : 66 in F_3) 60 green plants were grown as F_4 progenies in sand dishes. Here a trimeric segregation was obtained. The 60 progenies consisted of 34 normal-green, 5 trimerically segregating (numbers found: 2 : 118, 2 : 91, 1 : 121, 1 : 112 and 2 : 146), 12 di-, and 9 monomerically segregating progenies. The theoretical distribution would be: 35,2 : 7,6 : 11,5 : 5,7 for a trimeric ratio. χ^2 amounts to 2,863 and $0{,}50 > P > 0{,}30$, i. e. a good correspondence. At present we can only conclude that the *A. chinensis* strain used must carry *at least three Chlor* factors.

Chlorina × *A. sterilis* L. *s.str.* — Cross Nos. 506 and 507 from 1941 yielded two F_2 non-segregating progenies in 1943: 962 r 8 and r 9. The former (from 25 kernels sown) gave 9 full-grown green plants, the latter (from 33 kernels) gave 10 green plants. The 19 green plants from both F_2 progenies were thrashed and grown as F_3 in the field in 1944. Seeing that — as in the above-mentioned cross *chlorina* × *A. chinensis* —, the seed-setting of F_2 was very restricted, the F_3 progeny distribution could not satisfactorily be interpreted. The F_3 in question consisted of 10 non-segregating and 9 segregating progenies, the latter showing the ratios 3 : 47, 1 : 69, 1 : 23, 1 : 54, 5 : 55, 2 : 65, 5 : 13, 6 : 86, and 1 : 42 *chlorina* : green. From the first of the last-mentioned F_3 progenies (segregating 3 : 47) 39 green full-grown plants were procured for F_4 sand-dish growing in the laboratory. A trimeric segregation was then revealed: 25 green, 4 trimerically segregating (numbers found: 1 : 89, 2 : 128, 2 : 137 and 2 : 190), 7 di-, and 3 monomerically segregating progenies were counted. The theoretical numbers would be 22,9 : 5,0 : 7,4 : 3,7 for a 63 : 1-distribution. If the last two classes are reckoned together, we get $\chi^2 = 0{,}502$ and $0{,}80 > P > 0{,}70$, i. e. a good correspondence. At present we can merely conclude that the *A. sterilis* strain used must carry *at least three Chlor* factors.

Chlorina × *Dalslandshavre 29/100.* — Cross No. 326 from 1943 yielded three F_1 kernels, which were planted in the greenhouse in the winter of 1943—44 and grown in electrical light. Unfortunately, the three

resulting F_1 plants were weak and yielded merely 4, 12 and 10 kernels. The F_2, grown in the field in 1944, showed no segregation but was decidedly the result of a true cross. In F_3, grown in sand dishes in the laboratory in 1944—45, positive evidence of the existence of at least dimerically segregating F_3 progenies was procured. Furthermore, among 10 F_3 progenies from the same F_2 plant one segregated in 188 green : 3 *chlorina* and another segregated in 80 : 1, both indicating a trimeric ratio. Thus, Dalslandshavre 29/100 *carries at least two Chlor* factors.

Chlorina × *Förädlad Dalahavre.* — Cross No. 333 from 1941 yielded the three F_2 progenies 1943—962 r 30—32, segregating according to following tabulation:

Number of F_3 progeny	Kernels sown	Seedlings procured, altogether	Green ones	*Chlorina* recessives
r 30	227	199	194	5
r 31	238	224	221	3
r 32	100	95	94	1
	565	518	509	9

For a trimeric segregation the expected numbers are 509,9 : 8,1, i. e. a very good correspondence. For a supposed dimeric segregation we should expect 485,6 : 32,4, which gives a $\chi^2 = 18{,}028$ with $P < 0{,}001$. Since, however, the plots r 30—32 were attacked to some extent by the frit fly, it was recorded in the field notations that the strict classification of green and *chlorina* plants was difficult. If a number of recessives have thus been overlooked, a dimeric segregation might possibly have been recorded; unfortunately, an F_3 dish growing has not yet been undertaken. Thus, Förädlad Dalahavre *contains at least two Chlor* factors.

Chlorina × *Nidarhavre.* — Cross No. 310 from 1941 yielded the four F_2 progenies 1943—962 r 10—13, segregating as follows:

Number of F_3 progeny	Kernels sown	Seedlings procured, altogether	Green ones.	*Chlorina* recessives
r 10	283	267	264	3
r 11	234	207	200	7
r 12	178	152	151	1
r 13	61	54	52	2
	756	680	667	13

The expected numbers for a trimeric segregation are 669,4 : 10,6, i. e. a very close correspondence. For a 15 : 1 segregation we should

have the expected values 637,5 : 42,5; $\chi^2 = 21{,}842$ and $P < 0{,}001$. For the same reasons as in the F_2 of *chlorina* × Förädlad Dalahavre (see above) a slight attack of the frit fly in these plots may, however, have caused a disturbed classification in favour of the green plants, as was duly noted in the field book of 1943. Nidarhavre *contains at least two Chlor* factors; since no F_3 has yet been grown, the possibility of three *Chlor* factors must still be left an open question.

Chlorina × *Smålandshavre 29/37.* — Cross No. 363 from 1941 yielded a single F_2 progeny: 1943—962 r 29. From 205 sown kernels 195 seedlings were procured, comprising 193 green and 2 *chlorina*. For a 63 : 1 ratio the expectation is 191,95 : 3,05 with $0{,}70 > P > 0{,}50$. For a dimeric ratio the expected numbers would be 182,8 : 12,2 with $\chi^2 = 9{,}097$ and $0{,}01 > P > 0{,}001$. The segregation strongly indicates a trimeric ratio (no frit fly attacks observed, good germination). Since, however, no F_3 has yet been grown, we cannot with certainty assign more than *at least two Chlor* factors to the variety tested.

The results from the various *chlorina* crosses are summarized below.

3. DISCUSSION.

In Table 2 the results hitherto gained (in all 28 varieties analysed) are summarized in a scheme of evolution comprising varieties with three-, two- and monofactorial constitution in respect to *Chlor* factors. In the cross analysis only the total number of *Chlor* factor couples has been ascertained (*chlor* is the recessive factor according to ÅKERMAN and FRÖIER, 1941), not the specific *Chlor* set of each variety. However, on the basis of certain practical breeding crosses made at Svalöf between some of the strains tested in these experiments, indirect evidence can in some cases be obtained. Among the monofactorial Probsteier types at the bottom to the left in Table 2 Guldregn I (from which the f. *chlorina* arose as a spontaneous mutation) has the constitution *Chlor 1 Chlor 1 chlor 2 chlor 2 chlor 3 chlor 3.* Since Guldregn I has been crossed for practical breeding purposes with 0101, and no *chlorina* segregation was observed in the F_2, 0101 must have the same constitution. Likewise, in crosses 0101 × Ligowo I (which is practically identical with Ligowo II and cannot be supposed to differ in respect to the set of *Chlor* factors, the latter being merely a new élite stock from the old Ligowo I) no *chlorina* segregation was found. Hence Ligowo II must likewise have the same constitution.

Moreover, as in practical crosses Guldregn I × Seger and Ligowo II

× 0668 *chlorina* segregation was never observed, the varieties mentioned must carry *Chlor 1* together with either *Chlor 2* or *Chlor 3*. It is highly probable that also bifactorial varieties occur with *Chlor 2*

TABLE 2. *Total number of Chlor factors (Chlor 1, Chlor 2, Chlor 3) in various hexaploid oat strains. Varieties hitherto not fully investigated placed in brackets; cross-bred varieties not in italics.*

Total number of *Chlor* factors	Probsteier types	Central-Swedish black-hulled oats	Land oats from Central and South Sweden	North-Scandinavian early oats	Other varietes
3		*Fyrishavre* Klock II	*0691, Brun Ölandshavre*	*Thorshavre*	*Hedehavre* (*A. byzantina:* at least 3) (*A. chinensis:* at least 3) (*A. sterilis:* at least 3)
2	*Segerhavre*	*Stormogul I*	(*Smålandshavre* $^{29}/_{37}$: at least 2) (*Dalslandshavre* $^{29}/_{100}$: at least 2) (*Förädlad Dalahavre:* at least 2)	*0668* (north-Norwegian) Orion II (= Ligowo II × 0668) (*Nidar:* at least 2)	
1	*Guldregn I* *Abeds Nova* *0101* (Jaune g. à gr.) *Gul Naesgaardshavre* *Ligowo II* *Mansh. Binder* v. Lochow's Fläminggold		*Gotlandshavre*	*0660* (north-Finnish) *Mesdag*	*A. sativa* v. *aurea* (Turkestan) Cartier

together with *Chlor 3*: such strains giving a 63 : 1-segregation of *chlorina* recessives when crossed with Guldregn I. This would only be in strict analogy with the *lutescens* case (Ch. III), where the Abeds Nova oats have the constitution $l_s l_s\, L_p L_p\, L_n L_n$. Furthermore, monofactorial

strains with the two other possible *Chlor* factorial distributions must be supposed to exist, though not yet revealed (*chlor 1 chlor 1 Chlor 2 Chlor 2 chlor 3 chlor 3* and *chlor 1 chlor 1 chlor 2 chlor 2 Chlor 3 Chlor 3*). Finally, the three-factorial strains, of course, give no segregation, crossed with members of the two- or monofactorial groups. As for the varieties or species in brackets at the top to the right in Table 2 the conclusion from the cross analysis is that they carry »at least three *Chlor* factors» — it should, however, be emphasized that *there has been no indication whatever that these varieties carry more than three factors.*

Table 2 suggests some interesting conclusions as to the occurrence of phenotypically hidden *Chlor* factor mutation steps in hexaploid oats. If we assume the three-factorial constitution *Chlor 1 Chlor 1 Chlor 2 Chlor 2 Chlor 3 Chlor 3* as the phylogenetically primary one — which for several reasons seems quite justifiable —, we can trace the successive mutational steps down to the monofactorial stage — in the case of Guldregn I even as far as down to the monoheterozygote *Chlor 1 chlor 1* . . . , since the last mutational step was here phenotypically revealed. The number of investigated hexaploids is, of course, relatively limited — in proportion to the world-assortment available. The 28 strains tabulated will, however, be sufficient for the discussion.

All Probsteier types investigated except one (Seger) carry only a single *Chlor* factor couple. No trifactorial Probsteier variety has yet been found.

Among the black-hulled central-Swedish oats, on the other hand, no monofactorial strains have hitherto been found. The ecological differentiation between the Probsteier strains on the one hand, and the black-hulled central-Swedish varieties on the other, is thus accompanied also by a differentiation in the sets of *Chlor* factors. In Ch. III it is shown that quite reverse conditions exist in respect to the sets of *lutescens* factors (Fig. 2, p. 358): most Probsteier types carry three *L* factor couples, and all black-hulled central-Swedish oats carry only one *L* factor couple.

Among the various land-oats tested we trace the same mutational steps downwards to a monofactorial condition. Thus, 0691, Brun Ölandshavre, carries three *Chlor* factor couples, Gotlandshavre only one *Chlor* factor couple. As to the strains selected from Smålandshavre, Dalslandshavre and the variety Förädlad Dalahavre we can at present only conclude that they have not advanced very far in *chlorina* mutating, as they carry at least 2 (perhaps 3) *Chlor* factor couples. Among the north-Scandinavian early oats representatives of all factorial

stages are met with: Thorshavre with 3, 0668 with 2, and 0660 with 1 *Chlor* factor couples.

Among the systematically more distantly related hexaploid oats in the extreme right column the variety used of *A. sativa* v. *aurea* carries only 1 *Chlor* factor couple; *A. byzantina*, *chinensis* and *sterilis* are at the primary stage with all three *Chlor* factor couples still retained.

A transitional stage between the 3- and 1-factorial one can, of course, originate from other causes than successive mutational steps — for example, a 2-factorial oat variety, especially a recently cultivated and bred one, might also arise by means of crosses between two 1-factorial strains. A »rebuilding» of phylogenetically primary sets might thus take place. A three-factorial strain may be produced by crosses between a 2- and an 1-factorial strain, and so on, assuming the appropriate factorial constitution. Furthermore, by means of reduplication, e. g., »reverse repeats» according to BRIDGES (1937; in *Drosophila*) 2-factorial and 3-factorial strains might be produced from 1- and 2-factorial ones, respectively. Our knowledge of the occurrence of such duplications in plants still being extremely scanty, we cannot at present attach any definite significance to such phenomena in the discussion, but must content ourselves with bearing in mind their possibilities.

The objections thus raised against the hypothesis of successive *Chlor* factor mutation cannot, however, obscure its essential features. The facts gathered in regard to *lutescens* factor distribution further support the chief conclusions drawn. These conclusions are: (1) mutation of the homologous, polymeric *Chlor* factors has occurred repeatedly in the phylogeny of cultivated oats; (2) this successive mutating of the primarily most probably existing three *Chlor* factor couples has for some reason not advanced as far in the black-hulled central-Swedish oats as in the Probsteier varieties tested; (3) also as regards other hexaploid oats tested the same successive mutational steps can be traced; (4) the spontaneous mutation frequency *Chlor* → *chlor* must be very small since (a) so many market strains have been shown to be monofactorial and up to now merely one case of mutation to the recessive has been observed, and as (b) many trifactorial strains seem still to exist.

4. ADDENDUM: NOTE ON SOME PHYSIOLOGICAL PROPERTIES OF THE CHLORINA RECESSIVE.

These investigations were carried out in co-operation with Dr. GÖSTA ANDERSSON in the summer season of 1940. The apparatus used, designed by ANDERSSON and BOYSEN-JENSEN, is described by ANDERSSON (1944). The method permits a very

exact determination of the CO_2-production per unit leaf area and hour at the selected temperature (the latter may vary between + 1° and + 30° C. without affecting the exactness of the method). This method, however, demands a whole day for a single assimilating experiment with varying light intensities — this variation of intensity is necessary in order to reveal possible disturbances in the work of the stomatal mechanism — and thus one day must be spent on the *chlorina* mutation and the next day on the study of the normal mother strain, and so on.

The method further demands, if possible, equally large leaf areas. Thus, for convenient comparison, leaves of the same ontogenetical order should be used; furthermore, they should possess the same size and length. In the field material of 1940 about 5.000 *chlorina* plants (recessives) could be used for sampling purposes. Thus, very good facilities were available for procuring *chlorina* leaves conformable in size with normal Guldregn I leaves. Only the 4th, 5th and 6th leaves from field material were used in these experiments, as the leaves of lower order were at this time too old and might be supposed to involve experimental complications owing to partial withering, lacking turgor, stomatal disturbances, etc. In Table 3 the results of one such experiment (with the 5th leaf) are reported and diagrams given. The experiments so far carried out furnish us with certain interesting facts:

(1) The *chlorina* triple recessive shows a distinctly lower assimilation intensity *at medium to high light intensities* (5.000—30.000 »Lux»). Full sunlight in the middle of July at noon represents about 70.000 »Lux». In a field plot with green plant leaves the intensity of sunlight on such a day seldom reaches 30.000 »Lux» on the leaves of the investigated order. Thus, for intensities from 5.000 up to 30.000 »Lux» the 5th and 6th leaf show 25—35 % lower assimilation intensity of the *chlorina* recessive than of the normal Guldregn I.

(2) At low and very low light intensities (1.500—500 »Lux») the *chlorina* recessive at least equals — if not overweighs — the normal form in respect to assimilation intensity. This interesting feature of the chlorophyll-deficient form claims attention in future research work on chlorophyll physiology. There is an obvious indication of a somewhat higher assimilation energy of the *chlorina* leaves at intensities between 500 and 1.500 (2.000?) »Lux». It is, however, a fact that the respiration intensity is lower in the *chlorina* form than in the normal strain; hence the crossing points of the curves in Diagram 1. The present writer has in vain tried to carry out assimilation experiments with the sixth leaf of both forms at light intensities of 500, 1.000 and 1.500 »Lux», in order to get conclusive results. The capricious behaviour of the stomatal mechanism, however, complicated these experiments and completely obscured their results.

Anatomical studies on the development of the chloroplasts (regards the technique, see p. 365) have shown that the *chlorina* plants at first show fewer and smaller chloroplasts than the normal strain. Comparatively soon, however, even the first leaf has produced equally numerous and equally large chloroplasts as the corresponding leaf of the normal strain; the same was found for leaves of later order. Thus, the difference in assimilation intensity must chiefly be attributed to smaller quantities of active chlorophyll *a* and *b* available for photosynthesis.

TABLE 3. *Experiment with assimilation intensity of the 5th leaf of normal Guldregn I oats and of the chlorina mutation. Values of assimilation intensity procured from 8 o'clock to 13 o'clock.*

Leaf area used: for normal Guldregn I 40,44 cm² Temperature: 20° C.
for *chlorina* mutation 43,55 cm²

Day for experiment: $^3/_7$ 1940 (normal Guldregn I)
$^4/_7$ » (*chlorina* mutation)

Light intensity in »Lux» units according to photo-electric Lux-meter by D. Bercowitz & Sohn, Berlin. For further particulars as to the measurings of light intensity, see ANDERSSON (1944).

Light intensity »in Lux»:	CO_2-consumption per 50 cm² leaf area and 1 hour in mg for Normal Guldregn I	*chlorina*
0	— 0,74	— 0,55
2 000	+ 0,72	+ 0,74
10 000	+ 5,15	+ 3,49
15 000	+ 5,33	+ 4,51
20 000	+ 6,82	+ 5,00
30 000	+ 6,99	+ 5,46

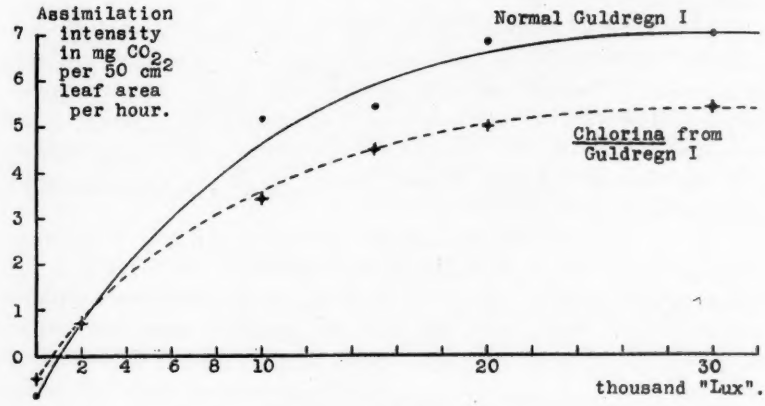

Diagram 1 (cf. Table 3).

III. THE DISTRIBUTION OF LUTESCENS FACTORS ON FIFTEEN HEXAPLOID OAT STRAINS.

1. CROSS COMBINATIONS. THEORETICAL SEGREGATION ALTERNATIVES.

In a report on the distribution of the three *lutescens* factors L_s, L_p and L_n on certain varieties of *A. sativa* (ÅKERMAN and FRÖIER, 1942) it was shown that Abeds Nova oats possessed the two polymeric, homologous factors L_p and L_n. The four central-Swedish black-hulled oats Klock II, Klock III, Stormogul I and Fyrishavre possessed the third, called L_s. (A correction: In the paper mentioned, p. 175, line 7 ff. from above, Fyrishavre has been omitted: this variety was also investigated in ÅKERMAN's earlier work and reported by him in 1922. It carries the same L factor as Klock II, III and Stormogul I, namely L_s). In further crosses, the monoheterozygote $L_s l_s\, l_p l_p\, l_n l_n$, isolated from the cross Klock II × yellow-stained monoheterozygote from the first cross

revealing *lutescens* segregation (Abeds Nova × Klock II), was hybridized with the following oat varieties:

0210, *A. orientalis* SCHREB. v. *tristis* AL. (pure line from Black Tartarian oats of English origin, selected by N. HJ. NILSSON, 1894); Cartier; Dalslandshavre 29/100; Ligowo II; v. Lochow's Fläminggold; v. Lochow's Gelbhafer (origin: cf. v. Lochow's Fläminggold, p. 313); Mansholt's Binder; Mesdag; Orion II and Seger. As to the origin of these strains, cf. Ch. II.

As the triple-recessive $l_s l_s\, l_p l_p\, l_n l_n$ cannot be used in this cross analysis, the demands on sufficient number of F_2 progenies are more pronounced in comparison with the case, e. g., in the *chlorina* crosses. In the crosses with the *lutescens* monoheterozygote the F_1 must, in fact, according to the possible alternatives (see below) contain two classes. For the sake of clearness a short survey of the various segregation possibilities is given below:

A. In crossing the monoheterozygote $L_s l_s\, l_p l_p\, l_n l_n$ to a three-factorial strain $L_s L_s\, L_p L_p\, L_n L_n$, F_1 will consist of 50 % $L_s l_s\, L_p l_p\, L_n l_n$ (triple-heterozygotes) and 50 % $L_s L_s\, L_p l_p\, L_n l_n$, *both phenotypically normal green.* Thus, in F_2, 50 % of the progenies must segregate in 63 green: 1 withering *lutescens* triple recessive; 50 % must remain constant green.

B: I. In crossing the monoheterozygote $L_s l_s\, l_p l_p\, l_n l_n$ to a two-factorial strain of the constitution $l_s l_s\, L_p L_p\, L_n L_n$ (the monoheterozygote has one of the *L* factors, the two-factorial strain the second and third) F_1 will consist of 50 % $L_s l_s\, L_p l_p\, L_n l_n$ (triple-heterozygotes) and 50 % $l_s l_s\, L_p l_p\, L_n l_n$ (double heterozygotes). In F_2 50 % of the progenies will segregate according to 63 : 1, the other 50 % according to 15 : 1. F_1 as before *homogeneous and phenotypically normal green.*

B: II. If the monoheterozygote $L_s l_s\, l_p l_p\, l_n l_n$ is hybridized with a two-factorial strain of the constitution $L_s L_s\, L_p L_p\, l_n l_n$ or $L_s L_s\, l_p l_p\, L_n L_n$ (the monoheterozygote has the factor L_s in common with the two-factorial variety) F_1 will consist of 50 % double heterozygotes ($L_s l_s\, L_p l_p\, l_n l_n$ or $L_s l_s\, l_p l_p\, L_n l_n$) and 50 % $L_s L_s\, L_p l_p\, l_n l_n$ or $L_s L_s\, l_p l_p\, L_n l_n$. F_2 will thus consist of 50 % 15 : 1-segregating progenies, and of 50 % constant green ones. F_1 as before *homogeneous and phenotypically normal green.*

C: I. If the monoheterozygote $L_s l_s\, l_p l_p\, l_n l_n$ is hybridized with a monofactorial variety *of the same factorial set*, although of course homozygous for L_s ($L_s L_s\, l_p l_p\, l_n l_n$), F_1 will consist of 50 % monoheterozygotes ($L_s l_s\, l_p l_p\, l_n l_n$) and 50 % monohomozygotes ($L_s L_s\, l_p l_p\, l_n l_n$). *The former are recognizable as they are temporarily*

yellow-stained; occasionally their classification is, however, disturbed by phenotypical variability. The latter are normal-green. Thus, F_2 will consist of 50 % 3 : 1-segregating progenies and 50 % constant green ones.

C: *II*. If the monoheterozygote $L_s l_s\, l_p l_p\, l_n l_n$ is hybridized with a monofactorial variety of the constitution $l_s l_s\, L_p L_p\, l_n l_n$ or $l_s l_s\, l_p l_p\, L_n L_n$, F_1 will consist of 50 % *yellow-stained monoheterozygotes* ($l_s l_s\, L_p l_p\, l_n l_n$ or $l_s l_s\, l_p l_p\, L_n l_n$), and 50 % double heterozygotes ($L_s l_s\, L_p l_p\, l_n l_n$ or $L_s l_s\, l_p l_p\, L_n l_n$), the latter normal-green. Thus, F_2 will consist of 50 % 3 : 1-segregating progenies and 50 % 15 : 1-segregating ones.

The genetical analysis hitherto made has not been advanced as far as to differentiate between the two sub-alternatives included in *B*: *II* and *C*: *II* above. In spite of this, the results have been summarized and presented below; a condensed survey is given in Fig. 2, p. 358.

2. GENETICAL RESULTS.

Lutescens monoheterozygote × *0210, A. orientalis* v. *tristis*. — Cross No. 340 from 1943 gave two kernels, grown as F_1 which was normal-green in the greenhouse in the winter of 1943—44. The two resulting F_2 field-progenies were very small: 15 and 13 plants respectively. No *lutescens* recessives were observed among these F_2 plants, but three yellow-stained monoheterozygotes were recorded in each progeny. F_3 growings in the laboratory of all the F_2 plants in sand dishes in 1944—45 gave the following results:

Number of F_2 progeny (field)	Number of F_3 dish progeny	Kernels sown	Seedlings procured, altogether	Green ones	Triple recessives	Mode of F_3 segregation
1944—962—r 47	1	159	158	157	1	63 : 1
	2	121	118	all	—	—
	3	124	121	all	—	—
	4	258	250	all	—	—
	5	131	112	88	24	3 : 1
	6	123	115	88	27	3 : 1
	7	225	215	163	52	3 : 1
	8	173	165	all	—	—
	9	169	160	all	—	—
	10	156	139	138	1	63 : 1
	11	212	201	191	10	15 : 1
	12	98	94	70	24	3 : 1
	13	135	130	all	—	—
	14	283	270	254	16	15 : 1
	15	169	159	all	—	—
1944—962—r 48	1	205	195	193	2	63 : 1
	2	154	142	112	30	3 : 1
	3	129	120	96	24	3 : 1

Number of F_2 progeny (field)	Number of F_3 dish progeny	Kernels sown	Seedlings procured, altogether	Green ones	Triple recessives	Mode of F_3 segregation
1944—962—r 48	4	225	218	all	—	—
	5	73	70	50	20	3 : 1
	6	162	159	150	9	15 : 1
	7	135	128	all	—	—
	8	20	15	11	4	3 : 1
	9	123	118	all	—	—
	10	176	170	134	36	3 : 1
	11	144	140	135	5	15 : 1
	12	175	169	129	40	3 : 1
	13	270	260	257	3	63 : 1

In spite of the limited number of F_3 progenies, each series, fortunately, unquestionably shows at least trimeric segregation (the families

Number of F_3 progeny	Seeds sown	Seedlings procured, altogether	Found: green/recessives	Expected: green/recessives	P-values	Mode of segregation
1	122	110	all green	—	—	—
2	174	160	» »	—	—	—
3	80	70	» »	—	—	—
4	116	101	» »	—	—	—
5	24	23	» »	—	—	—
6	50	41	» »	—	—	—
7	141	117	112 : 5	$109{,}7 : 7{,}3$	$0{,}50 > P > 0{,}30$	15 : 1
8	156	141	all green	—	—	—
9	96	89	» »	—	—	—
10	43	40	» »	—	—	—
11	49	40	» »	—	—	—
12	216	194	181 : 13	$181{,}9 : 12{,}1$	$0{,}80 > P > 0{,}70$	15 : 1
13	109	87	85 : 2	$85{,}6 : 1{,}4$	$0{,}70 > P > 0{,}50$	63 : 1
14	6	6	all green	—	—	—
15	7	5	» »	—	—	—
16	8	7	» »	—	—	—
17	59	58	57 : 1	$57{,}1 : 0{,}9$	$0{,}95 > P > 0{,}90$	63 : 1
18	68	61	all green	—	—	—
19	29	22	» »	—	—	—
20	76	71	» »	—	—	—
21	54	50	» »	—	—	—
22	145	137	135 : 2	$134{,}9 : 2{,}1$	$0{,}95 > P > 0{,}90$	63 : 1
23	151	140	all green	—	—	—
24	33	25	» »	—	—	—
25	75	68	» »	—	—	—
26	50	41	» »	—	—	—
27	166	135	» »	—	—	—
28	72	63	» »	—	—	—
29	206	159	» »	—	—	—
30	42	39	» »	—	—	—
31	291	197	183 : 14	$184{,}7 : 12{,}3$	$0{,}70 > P > 0{,}50$	15 : 1
32	477	450	all green	—	—	—
33	25	23	» »	—	—	—
34	156	124	112 : 12	$116{,}2 : 7{,}8$	$0{,}20 > P > 0{,}10$	15 : 1
35	58	41	all green	—	—	—

1 and 10 in the former, 1 and 13 in the latter represent beyond reasonable doubt trimeric segregations). But, owing to the unduly small F_1 generation it is impossible to differentiate between alternative *A* and *B*: *I* above. Thus, we can merely conclude that 0210 must contain *at least two L factors* ($L_pL_p\ L_nL_n$).

Lutescens monoheterozygote × *Cartier.* — Cross No. 399 from 1938 resulted in a single, normal-green F_1 plant, giving the F_2 progeny 1940—951 r 31. 65 sown kernels gave 51 plants among which were 1 withering recessive: 6 yellow-stained monoheterozygotes: 44 green. Theoretically, we would expect for a 63 : 1 ratio 0,8 : 4,8 : 45,4 with $\chi^2 = 0{,}393$ and $0{,}90 > P > 0{,}80$, i. e. a very good correspondence. For a 15 : 1 ratio we would expect the segregation 3,2 : 12,8 : 35,0 which gives $\chi^2 = 7{,}439$ and $0{,}05 > P > 0{,}02$ indicating a relatively significant deviation. F_3 dish growings of 35 *green* F_2 plants (the yellow-stained monoheterozygotes in F_2 thus omitted) gave the results in the table on p. 338 below.

Thus, the F_3 procured indisputably shows a distribution following a trimeric segregation of the F_2. As only one F_2 progeny was available (crosses repeated in 1941—1943 with Cartier have failed) we can merely conclude that Cartier must possess *at least the two lutescens* factors $L_pL_p\ L_nL_n$; it is not possible to choose either of the two alternatives *A* and *B*: *I* above as the most probable one.

Lutescens monoheterozygote × *Dalslandshavre 29/100.* — Cross No. 377 from 1942 resulted in three F_1 plants which were normal-green. They grew in the open field in 1943 and yielded three F_2 progenies according to following tabulation:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Green ones	Yellow-stained monoheterozygotes	Triple *lutescens* recessives
1944—967—r 27	241	180	all	—	—
r 28	336	256	all	—	—
r 29	331	205	144	42	19

For the segregation of r 29 according to a 15 : 1 ratio the expected numbers would be 140,9 : 51,3 : 12,8 with $\chi^2 = 4{,}757$ and $0{,}10 > P > 0{,}05$; i. e. in 5—10 % of all cases such an F_2 segregation could be expected. A segregation according to a supposed trimeric ratio would demand the expected values 182,6 : 19,2 : 3,2. For the calculation of χ^2 the last classes are taken together. We then get $\chi^2 = 74{,}676$ with $P < 0{,}001$. Thus, a dimeric segregation is the most probable. The F_3 analysis in sand dishes confirms the supposition of a dimeric ratio definitely, as shown below:

Number of F_3 progeny	Kernels sown	Seedlings procured, altogether	Green ones	*Lutescens* recessives	Most probable mode of segregation
1	126	120	all	—	—
2	158	152	all	—	—
3	101	95	90	5	15 : 1
4	144	129	120	9	15 : 1
5	73	65	51	14	3 : 1
6	200	195	all	—	—
7	162	152	all	—	—
8	137	130	all	—	—
9	87	83	all	—	—
10	144	140	all	—	—
11	83	80	all	—	—
12	46	44	all	—	—
13	97	92	68	24	3 : 1
14	66	60	all	—	—
15	105	100	95	5	15 : 1
16	13	11	8	3	3 : 1
17	71	69	all	—	—
18	20	19	all	—	—
19	95	90	all	—	—
20	72	65	62	3	15 : 1
21	69	68	63	5	15 : 1
22	98	96	all	—	—
23	87	82	77	5	15 : 1
24	51	48	35	13	3 : 1
25	67	63	57	6	15 : 1
26	148	145	139	6	15 : 1
27	92	92	89	3	15 : 1 ?
28	81	79	76	3	15 : 1 ?
29	122	115	all	—	—
30	74	70	66	4	15 : 1
31	21	21	all	—	—
32	192	182	174	8	15 : 1
33	79	75	60	15	3 : 1
34	65	63	60	3	15 : 1
35	80	76	all	—	—
36	152	150	140	10	15 : 1
37	61	58	all	—	—
38	77	75	all	—	—
39	108	103	all	—	—
40	152	142	all	—	—
41	130	120	114	6	15 : 1
42	168	165	150	15	15 : 1
43	90	85	all	—	—
44	87	83	all	—	—
45	35	34	23	11	3 : 1
46	128	120	95	25	3 : 1
47	106	101	94	7	15 : 1
48	142	135	127	8	15 : 1
49	94	83	79	4	15 : 1
50	110	108	105	3	15 : 1 ?
51	40	39	all	—	—
52	50	50	47	3	15 : 1
53	65	64	62	2	15 : 1 ?
54	116	105	86	19	3 : 1
55	72	70	all	—	—
56	104	100	95	5	15 : 1
57	24	21	15	6	3 : 1
58	15	14	11	3	3 : 1

These 58 plants were taken at random from the green *and* yellow-stained plants in F_2, as it was considered opportune not to rely fully on the strict classification of the yellow-stained monoheterozygotes. The four progenies 27, 28, 50 and 53 must be supposed to have segregated dimerically in spite of their rather deviating ratios, as F_2 gives no real reason for supposing a trimeric segregation. If we thus count the four families mentioned as dimerically segregating ones and calculate the probability of a dimeric distribution of the F_3 progenies, we have the expected numbers 27,0 green : 15,5 di- : 15,5 monomerically segregating progenies. Found: 25 : 23 : 10. χ^2 amounts to 5,729 and $0{,}10 > P > 0{,}05$, i. e. no significant deviation from the 15 : 1 hypothesis. According to a supposed 63 : 1 segregation, we would get the expected values 34,1 : 7,4 : 11,0 : 5,5. Now, if we count the four »critical progenies» above as 63 : 1-segregating, we nevertheless obtain $\chi^2 = 13{,}491$ and $0{,}01 > P > 0{,}001$ for the trimerical segregation hypothesis, i. e. a rather significant deviation. If we count the four progenies as 15 : 1-segregating ones (as justified by the F_2 generation) we get $\chi^2 = 23{,}333$ and $P < 0{,}001$ for the trimerical segregation hypothesis. Thus, the dimerical segregation of the F_2 is indisputable. Now, differentiation must be made between the two alternatives *B*: *I* and *B*: *II* (p. 336). In *B*: *I* half of the F_2 segregates trimerically and the other half dimerically; in *B*: *II* one half segregates dimerically and the other half shows no segregation at all. Since both F_2 progenies 1944—967—r 27 and r 28 were the result of true crosses and both showed neither *lutescens* recessives nor yellow-stained monoheterozygotes, we have to consider their probability of representing a trimeric segregation with 0 recessives and 0 yellow-stained monoheterozygotes. The 436 seedlings (r 27 and r 28 taken together), according to a supposed 63 : 1-segregation would have segregated in a ratio 388,3 : 40,9 : 6,8. χ^2 then amounts to 53,560 and $P < 0{,}001$. Thus, beyond reasonable doubt the F_2 progenies in question do not show any trimeric segregation, and consequently alternative *B*: *II* is the valid one. *Dalslandshavre 29/100 is bifactorial* and has the genetical constitution $L_sL_s\,L_pL_p\,l_nl_n$ *or* $L_sL_s\,l_pl_p\,L_nL_n$; the analysis has not yet been carried so far as to differentiate between the last two possibilities. This is, however, the first new bifactorial variety met with after the detection of the bifactorial Abeds Nova, the line 29/100 from Dalslandshavre thus possessing a *lutescens* factor set distinctly different from that of Abeds Nova. In the discussion we will revert to these facts.

Lutescens monoheterozygote × *Ligowo II.* — Cross Nos. 175 and 177 from 1939 yielded two normal-green F_1 plants which in 1941 gave

the two F_2 progenies 952—r 39 and r 40. The first (r 39) gave 636 seedlings from 912 sown kernels: all seedlings were normal-green and no yellow-stained monoheterozygotes could be observed. No 63 : 1-segregation could be supposed in this r 39, since the P-value for such a segregation would be less than 0,001. The latter progeny, r 40 (kernels sown: 420; seedlings procured: 309), segregated in 277 green : 28 monoheterozygotes : 4 *lutescens* recessives. For a trimeric segregation we should expect the numbers 275,2 : 29,0 : 4,8 with $\chi^2 = 0{,}490$ and $0{,}80 > P > 0{,}70$, i. e. a very good correspondence. From the F_2 generation 89 *green* plants were thrashed and grown in dishes as F_3. Among these 89 plants 6 were found to be wrongly marked as normal-green F_2 plants — an illustration of the difficulties sometimes encountered in exact classifying of the monoheterozygotes, despite the greatest care. The results from the F_3 growings are tabulated below (pp. 343—344).

Thus, in this F_3 the 6 progenies from monoheterozygotes, wrongly classified as green plants can be omitted. We then get 83 progenies: 52 green, 12 tri- and 19 dimerically segregating. Corresponding theoretical numbers would be (per 57 we have 37 : 8 : 12) 53,9 : 11,6 : 17,5. $\chi^2 = 0{,}209$ and $0{,}95 > P > 0{,}90$, i. e. a very good correspondence. A supposed tetrameric segregation would give the expected values (per 247 we have 175 green : 16 tetra- : 32 tri- : 24 dimerically segregating F_3 progenies) 64,2 green + tetra- : 10,8 tri- : 8,0 dimerically segregating progenies. χ^2 would amount to 17,577 and $P < 0{,}001$. The F_3 distribution indisputably represents a trimeric segregation. An alternative not discussed before (pp. 336 ff.) is that of *supposing the existence of a fourth L factor*, yet unknown. The monoheterozygote used in all these crosses would then have to be written $L_s l_s\, l_p l_p\, l_n l_n\, l_x l_x$ where L_x is the entirely hypothetical — and, for many reasons, most probably non-existent — fourth *lutescens* factor. If this monoheterozygote is crossed with a supposed four-factorial strain $L_s L_s\, L_p L_p\, L_n L_n\, L_x L_x$, F_1 must consist of 50 % quadruple-heterozygotes $L_s l_s\, L_p l_p\, L_n l_n\, L_x l_x$ and 50 % $L_s L_s\, L_p l_p\, L_n l_n\, L_x l_x$, both constant green. F_2 will consist of 50 % 255 : 1-segregating progenies and 50 % constant green. Such an F_2 decidedly does not fit the F_2 found here; it could thus be concluded that Ligowo II carries the three *L* factors L_s, L_p *and* L_n, *no more and no less than these three.*

Lutescens monoheterozygote × *v. Lochow's Fläminggold.* — Cross Nos. 238, 239, 241 and 242 (all true crosses) from 1940 yielded four F_2 progenies in 1942 according to the table on page 344 below (F_1 quite normal-green).

Number of F_3 progeny	Kernels sown	Seedlings procured, altogether	Found: green/*lutescens*	Expected: green/*lutescens*	P-values	Mode of segregation
1	84	57	47 : 10	42,7 : 14,3	$0{,}20 > P > 0{,}10$	3 : 1 [1]
2	69	58	all green	—	—	—
3	68	46	43 : 3	43,1 : 2,9	$0{,}98 > P > 0{,}95$	15 : 1
4	47	43	40 : 3	40,3 : 2,7	$0{,}90 > P > 0{,}80$	15 : 1
5	45	31	all green	—	—	—
6	15	9	» »	—	—	—
7	97	86	84 : 2	84,7 : 1,3	$0{,}70 > P > 0{,}50$	63 : 1
8	86	61	58 : 3	57,2 : 3,8	$0{,}70 > P > 0{,}50$	15 : 1
9	67	58	54 : 4	54,4 : 3,6	$0{,}90 > P > 0{,}80$	15 : 1
10	72	60	all green	—	—	—
11	73	59	» »	—	—	—
12	108	81	» »	—	—	—
13	73	60	» »	—	—	—
14	117	101	» »	—	—	—
15	89	65	» »	—	—	—
16	99	82	» »	—	—	—
17	154	120	» »	—	—	—
18	113	80	» »	—	—	—
19	57	44	41 : 3	41,2 : 2,8	$0{,}70 > P > 0{,}50$	15 : 1
20	269	75	71 : 4	70,3 : 4,7	$0{,}80 > P > 0{,}70$	15 : 1
21	105	82	all green	—	—	—
22	42	10	» »	—	—	—
23	49	34	» »	—	—	—
24	78	25	18 : 7	18,7 : 6,3	$0{,}80 > P > 0{,}70$	3 : 1 [1]
25	81	61	58 : 3	57,2 : 3,8	$0{,}70 > P > 0{,}50$	15 : 1
26	82	48	36 : 12	36,0 : 12,0	$0{,}99 > P > 0{,}98$	3 : 1 [1]
27	100	82	all green	—	—	—
28	120	17	» »	—	—	—
29	117	67	66 : 1	65,95 : 1,05	$0{,}98 > P > 0{,}95$	63 : 1
30	94	42	39 : 3	39,2 : 2,8	$0{,}95 > P > 0{,}90$	15 : 1
31	78	55	54 : 1	54,2 : 0,8	$0{,}90 > P > 0{,}80$	63 : 1
32	55	42	40 : 2	39,4 : 2,6	$0{,}90 > P > 0{,}80$	15 : 1
33	94	45	35 : 10	33,7 : 11,3	$0{,}70 > P > 0{,}50$	3 : 1 [1]
34	79	53	48 : 5	49,7 : 3,3	$0{,}50 > P > 0{,}30$	15 : 1
35	75	33	31 : 2	30,9 : 2,1	$0{,}95 > P > 0{,}90$	15 : 1
36	51	25	all green	—	—	—
37	106	27	» »	—	—	—
38	191	109	103 : 6	102,2 : 6,8	$0{,}80 > P > 0{,}70$	15 : 1
39	76	52	51 : 1	51,2 : 0,8	$0{,}90 > P > 0{,}80$	63 : 1
40	194	160	158 : 2	157,5 : 2,5	$0{,}80 > P > 0{,}70$	63 : 1
41	114	63	all green	—	—	—
42	130	96	» »	—	—	—
43	105	48	» »	—	—	—
44	54	29	» »	—	—	—
45	140	97	» »	—	—	—
46	69	55	» »	—	—	—
47	54	50	49 : 1	49,2 : 0,8	$0{,}90 > P > 0{,}80$	63 : 1
48	67	50	all green	—	—	—
49	59	25	23 : 2	23,4 : 1,6	$0{,}80 > P > 0{,}70$	15 : 1
50	69	55	all green	—	—	—
51	105	83	» »	—	—	—
52	69	38	» »	—	—	—
53	52	40	» »	—	—	—

Number of F_3 progeny	Kernels sown	Seedlings procured, altogether	Found: green/*lut-escens*	Expected: green/*lut-escens*	P-values	Mode af segrega-tion
54	141	79	78: 1	77,8 : 1,2	$0{,}90 > P > 0{,}80$	63 : 1
55	81	48	all green	—	—	—
56	90	64	» »	—	—	—
57	83	61	» »	—	—	—
58	88	63	62: 1	62,02 : 0,98	$0{,}99 > P > 0{,}98$	63 : 1
59	85	65	all green	—	—	—
60	81	70	» »	—	—	—
61	96	75	70: 5	70,3 : 4,7	$0{,}90 > P > 0{,}80$	15 : 1
62	231	186	all green	—	—	—
63	121	99	» »	—	—	—
64	27	17	» »	—	—	—
65	109	83	» »	—	—	—
66	102	62	» »	—	—	—
67	81	32	25: 7	24,0 : 8,0	$0{,}70 > P > 0{,}50$	3 : 1 [1]
68	182	150	all green	—	—	—
69	90	71	65: 6	66,6 : 4,4	$0{,}50 > P > 0{,}30$	15 : 1
70	186	138	all green	—	—	—
71	92	59	» »	—	—	—
72	87	54	» »	—	—	—
73	43	43	» »	—	—	—
74	64	58	57: 1	57,1 : 0,9	$0{,}95 > P > 0{,}90$	63 : 1
75	82	69	68: 1	67,9 : 1,1	$0{,}95 > P > 0{,}90$	63 : 1
76	57	43	all green	—	—	—
77	57	52	51: 1	51,2 : 0,8	$0{,}90 > P > 0{,}80$	63 : 1
78	84	63	59: 4	59,1 : 3,9	$0{,}98 > P > 0{,}95$	15 : 1
79	136	112	100 : 12	105,0 : 7,0	$0{,}10 > P > 0{,}05$	15 : 1
80	65	33	all green	—	—	—
81	43	32	22 : 10	24,0 : 8,0	$0{,}50 > P > 0{,}30$	3 : 1 [1]
82	201	180	all green	—	—	—
83	78	71	57: 4	57,2 : 3,8	$0{,}95 > P > 0{,}90$	15 : 1
84	55	38	all green	—	—	—
85	101	62	» »	—	—	—
86	66	53	51: 2	49,7 : 3,3	$0{,}50 > P > 0{,}30$	15 : 1
87	59	52	all green	—	—	—
88	100	83	» »	—	—	—
89	135	76	75: 1	74,8 : 1,2	$0{,}90 > P > 0{,}80$	63 : 1

It was noted in the field-books that the classification of the mono-heterozygotes was somewhat difficult; probably too few have been ob-

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Green ones	Yellow-stained mono-heterozygotes	Triple *lutescens* recessives
1942—941—r 56	141	130	all	—	—
r 57	182	172	all	—	—
r 58	208	180	all	—	—
r 59	150	136	131	3	2

[1] These 3 : 1-segregating progenies were wrongly marked as normal-green F_2 plants.

served. If we, therefore, count the green and yellow-stained plants together, we get 134 : 2; expected for a trimeric ratio is 133,9 : 2,1, i. e. a very close conformity. The growing of F_3 in sand dishes was undertaken as regards no less than 122 *green* F_2 plants. For lack of space, detailed tabulation of the results will be omitted here. 10.155 seedlings were procured and counted, corresponding to an average of 83,2 germinated seedlings per F_3 family. 5 monoheterozygotes wrongly classified as green plants were revealed by their monomeric segregation in F_3. The other progenies were distributed as follows: 88 green, 8 tri- and 21 dimerically segregating. The expected numbers would be for a 63 : 1 ratio: 76,0 : 16,4 : 24,6. $\chi^2 = 6{,}724$ and $0{,}05 > P > 0{,}02$, thus indicating a significant difference from the 63 : 1-hypothesis. The number of trimerically segregating F_3 families is obviously too small, owing to the relatively limited average seedling number per dish (83,2). We are therefore justified in counting together the classes green and 63 : 1-segregating families, and thus get the ratio 96 : 21. Expected is 92,4 : 24,6. χ^2 now amounts to 0,667 and $0{,}50 > P > 0{,}33$, i. e. a good correspondence with the 63 : 1-hypothesis. A tetrameric distribution would require the expected numbers 105,6 (green + tetra- + trimerically segregating families) to 11,4 dimerically segregating ones. This gives $\chi^2 = 8{,}957$ and $0{,}01 > P > 0{,}001$, and is thus highly improbable. Beyond reasonable doubt v. Lochow's Fläminggold *must thus contain the three factors* L_s, L_p *and* L_n (alternative *A*), as the three F_2 progenies r 56—r 58 cannot reasonably be 63 : 1-segregating. On account of the definitively proved trimeric segregation of the F_2 progeny r 59, the progenies r 56—r 58 could not even be supposed to segregate in the ratio of 255 : 1, as this does not fit any possible segregation alternative.

Lutescens monoheterozygote × *v. Lochow's Gelbhafer.* — Cross No. 384 from 1942 yielded three F_2 progenies in 1944 according to the following tabulation (F_1 was quite normal-green):

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Green ones	Yellow-stained monohete-rozygotes	Triple *lutescens* reces-sives
1944—967—r 30	577	541	all	—	—
r 31	118	102	88	10	4
r 32	140	119	102	13	4

The two segregating F_2 progenies counted together give the ratio 190 : 23 : 8. For a 63 : 1-segregation we should expect the theoretical numbers 196,8 : 20,7 : 3,5. If the last two classes are counted together

we get $\chi^2 = 2{,}146$ and $0{,}20 > P > 0{,}10$, i. e. a fair correspondence. From r 31 60 F_2 plants were thrashed (green + yellow-stained, as in this case the short marking-sticks were taken away from the yellow-stained plants before harvest) and grown as 60 F_3 dish families. The results were:

Number of F_3 progeny	Kernels sown	Seedlings procured, altogether	Green ones	*Lutescens* recessi-ves	Most probable mode of segregation
1	104	97	77	20	3 : 1
2	66	63	all	—	—
3	212	203	193	10	15 : 1
4	52	50	34	16	3 : 1
5	65	64	63	1	63 : 1
6	81	79	74	5	15 : 1
7	130	125	all	—	—
8	87	80	all	—	—
9	129	120	all	—	—
10	79	60	all	—	—
11	104	100	all	—	—
12	141	130	121	9	15 : 1
13	104	93	all	—	—
14	9	9	all	—	—
15	108	90	74	16	3 : 1
16	61	58	all	—	—
17	88	80	76	4	15 : 1
18	91	86	85	1	63 : 1
19	93	89	84	5	15 : 1
20	124	118	all	—	—
21	81	76	all	—	—
22	135	132	127	5	15 : 1
23	78	75	71	4	15 : 1
24	92	90	88	2	63 : 1
25	105	103	101	2	63 : 1
26	115	109	all	—	—
27	150	140	all	—	—
28	135	129	all	—	—
29	86	82	77	5	15 : 1
30	31	29	all	—	—
31	59	57	all	—	—
32	133	125	123	2	63 : 1
33	96	93	all	—	—
34	77	72	all	—	—
35	130	121	all	—	—
36	92	83	all	—	—
37	101	95	all	—	—
38	65	64	all	—	—
39	50	43	31	12	3 : 1
40	88	81	all	—	—
41	81	76	all	—	—
42	93	90	all	—	—
43	113	103	all	—	—
44	128	121	113	8	15 : 1
45	122	115	all	—	—
46	71	65	all	—	—
47	72	68	63	5	15 : 1
48	99	94	87	7	15 : 1
49	44	41	all	—	—

Number of F_3 progeny	Kernels sown	Seedlings procured, altogether	Green ones	*Lutescens* recessi-ves	Most probable mode of segregation
50	98	92	all	—	—
51	61	58	45	13	3 : 1
52	22	21	20	1	15 : 1
53	159	140	all	—	—
54	141	135	133	2	63 : 1
55	27	25	all	—	—
56	73	68	56	12	3 : 1
57	72	68	all	—	—
58	109	90	all	—	—
59	90	80	63	17	3 : 1
60	204	193	189	4	63 : 1

Thus, 34 green, 7 tri-, 12 di- and 7 monomerically segregating F_3 families were found. The theoretical ratio (for a 63 : 1-distribution) would be $35{,}3 : 7{,}6 : 11{,}4 : 5{,}7$ with $\chi^2 = 0{,}423$ and $0{,}95 > P > 0{,}90$, i. e. an excellent correspondence. For a supposed tetrameric segregation we should expect the ratio $45{,}0 : 7{,}5 : 7{,}5$ (green + tetra- : tri- : di- + monomerically segregating progenies). Corresponding found numbers are 34 : 7 : 19 with $\chi^2 = 20{,}356$ and $P < 0{,}001$. The F_2 progeny r 31 is thus indisputably 63 : 1-segregating; the other, r 32, was not tested in an F_3 analysis, but must necessarily also be trimerically segregating since, e. g., a supposed 15 : 1-segregation of this r 32 F_2 progeny does not fit any possible alternative; the r 30 F_2 progeny can not reasonably be 63 : 1-segregating. No other alternative than *A* is possible. Thus, v. Lochow's Gelbhafer carries the *three lutescens factors* L_s, L_p *and* L_n.

Lutescens monoheterozygote × *Mansholt's Binder.* — Cross No. 383 from 1938 yielded a single normal-green F_1 plant, which gave the F_2 progeny 1940—951 r 20. From 119 kernels sown, 99 seedlings were procured. Among them 90 were green, 7 yellow-stained monoheterozygotes and 2 withering triple recessives. For a supposed 63 : 1 ratio we would theoretically expect $88{,}2 : 9{,}3 : 1{,}5$ with $\chi^2 = 0{,}773$ and $0{,}70 > P > 0{,}50$, i. e. a good correspondence. 75 F_2 plants, marked as normal-green, were thrashed and grown as F_3 in sand dishes. This time no wrongly classified monoheterozygotes were revealed, as is obvious from the tabulation of the F_3 segregations on pp. 348—349.

In this F_3 thus 49 constant green, 7 tri- and 19 dimerically segregating progenies were found. Corresponding expected numbers (for a 63 : 1 segregation) would be $48{,}7 : 10{,}5 : 15{,}8$ with $\chi^2 = 1{,}817$ and $0{,}50 > P > 0{,}30$, i. e. a good correspondence. For a supposed tetrameric segregation we should expect the ratio $58{,}0$ green + tetra- : $9{,}7$ tri- : $7{,}3$ dimerically segregating progenies. χ^2 then amounts to $20{,}900$ and $P < 0{,}001$.

Progeny number of F_3	Number of seeds sown	Number of germinated seeds	Found: green/*lutescens*	Expected: green/*lutescens*	P-values	Mode of segregation
1	18	18	all green	—	—	—
2	4	3	» »	—	—	—
3	94	91	» »	—	—	—
4	100	95	89 : 6	89,1 : 5,9	$0{,}98 > P > 0{,}95$	15 : 1
5	120	115	all green	—	—	—
6	140	130	» »	—	—	—
7	54	50	» »	—	—	—
8	435	410	» »	—	—	—
9	108	101	» »	—	—	—
10	36	31	» »	—	—	—
11	64	60	» »	—	—	—
12	97	91	» »	—	—	—
13	195	180	» »	—	—	—
14	22	20	» »	—	—	—
15	154	139	» »	—	—	—
16	35	22	20 : 2	20,6 : 1,4	$0{,}70 > P > 0{,}50$	15 : 1
17	75	67	63 : 4	62,8 : 4,2	$0{,}95 > P > 0{,}90$	15 : 1
18	103	91	all green	—	—	—
19	170	159	» »	—	—	—
20	21	20	» »	—	—	—
21	57	51	» »	—	—	—
22	30	29	» »	—	—	—
23	94	91	84 : 7	85,3 : 5,7	$0{,}70 > P > 0{,}50$	15 : 1
24	186	174	168 : 6	163,1 : 10,9	$0{,}20 > P > 0{,}10$	15 : 1
25	384	371	all green	—	—	—
26	107	100	» »	—	—	—
27	112	103	» »	—	—	—
28	80	76	» »	—	—	—
29	115	109	» »	—	—	—
30	68	65	» »	—	—	—
31	61	57	54 : 3	53,4 : 3,6	$0{,}80 > P > 0{,}70$	15 : 1
32	113	105	all green	—	—	—
33	252	249	» »	—	—	—
34	116	114	104 : 10	106,9 : 7,1	$0{,}30 > P > 0{,}20$	15 : 1
35	53	50	all green	—	—	—
36	129	120	119 : 1	118,1 : 1,9	$0{,}70 > P > 0{,}50$	63 : 1
37	8	8	7 : 1	7,5 : 0,5	$0{,}50 > P > 0{,}30$	15 : 1
38	120	115	all green	—	—	—
39	43	43	40 : 3	40,3 : 2,7	$0{,}90 > P > 0{,}80$	15 : 1
40	523	470	458 : 12	462,7 : 7,3	$0{,}10 > P > 0{,}05$	63 : 1
41	81	75	all green	—	—	—
42	177	169	» »	—	—	—
43	10	10	» »	—	—	—
44	94	89	88 : 1	87,6 : 1,4	$0{,}80 > P > 0{,}70$	63 : 1
45	229	225	224 : 1	221,4 : 3,6	$0{,}20 > P > 0{,}10$	63 : 1
46	148	143	133 : 10	134,1 : 8,9	$0{,}80 > P > 0{,}70$	15 : 1
47	142	138	all green	—	—	—
48	76	71	» »	—	—	—
49	51	47	» »	—	—	—
50	178	165	164 : 1	162,4 : 2,6	$0{,}50 > P > 0{,}30$	63 : 1
51	112	101	95 : 6	94,7 : 6,3	$0{,}95 > P > 0{,}90$	15 : 1
52	183	170	all green	—	—	—
53	57	51	48 : 3	47,8 : 3,2	$0{,}95 > P > 0{,}90$	15 : 1

Progeny number of F_2	Number of seeds sown	Number of germinated seeds	Found: green/*lutescens*	Expected: green/*lutescens*	P-values	Mode of segregation
54	39	33	all green	—	—	—
55	65	53	» »	—	—	—
56	22	21	» »	—	—	—
57	77	71	» »	—	—	—
58	98	91	» »	—	—	—
59	29	28	» »	—	—	—
60	191	181	172 : 9	169,7 : 11,3	$0{,}50 > P > 0{,}30$	15 : 1
61	263	256	240 : 16	240,0 : 16,0	$0{,}99 > P > 0{,}98$	15 : 1
62	103	99	all green	—	—	—
63	154	146	» »	—	—	—
64	70	56	52 : 4	52,5 : 3,5	$0{,}80 > P > 0{,}70$	15 : 1
65	109	101	all green	—	—	—
66	204	182	179 : 3	179,2 : 2,8	$0{,}95 > P > 0{,}90$	63 : 1
67	163	142	136 : 6	133,1 : 8,9	$0{,}50 > P > 0{,}30$	15 : 1
68	125	120	all green	—	—	—
69	57	52	47 : 5	48,7 : 3,3	$0{,}50 > P > 0{,}30$	15 : 1
70	68	61	57 : 4	57,2 : 3,8	$0{,}95 > P > 0{,}90$	15 : 1
71	145	136	all green	—	—	—
72	68	65	» »	—	—	—
73	175	163	» »	—	—	—
74	55	48	44 : 4	45,0 : 3,0	$0{,}70 > P > 0{,}50$	15 : 1
75	129	123	120 : 3	121,1 : 1,9	$0{,}50 > P > 0{,}30$	63 : 1

The F_2 progeny r 20 is indisputably trimerically segregating. Now, as no more F_2 progeny was available and repeated crosses in 1938 and 1939 unfortunately failed, there was no possibility so far to differentiate between the alternatives *A* and *B*: *I* above — the only two possible ones. If, however, *B*: *I* was the valid one (the constitution of Mansholt's Binder thus $l_s l_s L_p L_p \cdot L_n L_n$) crosses with Klock II (formula: $L_s L_s l_p l_p l_n l_n$) must needs reveal a 63 : 1 segregation. By means of this circuit alternative *B*: *I* could be discarded: Crosses Nos. 311, 312, 315, 316, 319 and 320 from 1943 between Mansholt's Binder and Klock II, carefully controlled gave an F_1 which was grown in the greenhouse in the winter of 1943—44. The F_1 plants were very weakly developed and produced few kernels each. F_2 was grown in the field in 1944 and consisted of 21 normal-green F_2 progenies with 23, 8, 12, 15, 12, 11, 15, 8, 10, 3, 12, 15, 4, 5, 12, 5, 6, 15, 11, 9 and 3 germinated seedlings, i. e. together 214 (the total number of kernels sown was 263). Could these 214 F_2 plants possibly represent a 63 : 1 segregation? Great care was taken in studying the seedlings during their earlier development so as to record possibly occurring yellow-stained monoheterozygotes but none were observed. Thus, against the found numbers 214 : 0 : 0 we have to contrast the theoretically expected ratio 190,6 : 20,1 : 3,3, which gives

$\chi^2 = 26{,}372$ and $P < 0{,}001$. Beyond reasonable doubt the F_2 progenies procured are thus not trimerically segregating; alternative *A* is the valid one. Mansholt's Binder carries the *three L factors* L_s, L_p *and* L_n.

Lutescens monoheterozygote × *Mesdag.* — Cross Nos. 412, 413 and 415 from 1938 gave fully normal-green F_1 plants, yielding 7 F_2 progenies in 1940. Their segregation is tabulated below:

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Green ones	Yellow-st. monohe-teroz.	*Lutescens* recessi-ves
1940—951—r 51	19	14	11	2	1
r 52	149	130	all	—	—
r 53	155	102	all	—	—
r 54	109	91	all	—	—
r 55	152	128	all	—	—
r 56	38	38	18	17	3
r 57	207	176	141	19	16

The F_1 and F_2 control of the crosses was very careful. If we count the three segregating F_2 progenies together, we find the ratio 170 : 38 : 20. Expected theoretically for a dimeric ratio would be $156{,}8 : 57{,}0 : 14{,}2$. Since obviously some monoheterozygotes have been wrongly classified as green plants we may advantageously count together the two first classes, and then get $\chi^2 = 2{,}526$ with $0{,}20 > P > 0{,}10$, i. e. a fair correspondence. A trimeric segregation would demand theoretically $224{,}4 : 3{,}6$, which gives $\chi^2 = 75{,}910$ and $P < 0{,}001$. A monomeric segregation is equally improbable ($\chi^2 = 32{,}023$; $P < 0{,}001$). The four non-segregating F_2 progenies r 52—r 55, when reckoned together, represent 451 wholly green seedlings. Any hidden 63 : 1 segregation among these progenies cannot reasonably be supposed. As neither the segregating three F_2 progenies singly nor their summated segregations give any reason to suppose anything but dimeric segregation of these progenies, alternative *B*: *II* is thus the valid one. Mesdag therefore — just as Dalslandshavre 29/100 above — must have the constitution $L_sL_s\,L_pL_p\,l_nl_n$ *or* $L_sL_s\,l_pl_p\,L_nL_n$. Thus, a second new variety is found — after Abeds Nova — with a bifactorial *lutescens* set like the latter oat variety but with only one *L* factor in common with Abeds Nova, the other factor in common with, for instance, Klock II. Cf. Discussion, p. 359.

Lutescens monoheterozygote × *Orion II.* — Cross No. 410 from 1938 gave a single, normal-green F_1 plant, yielding 667 seeds which were sown in 1940 as the F_2 progeny 1940—951 r 31. 580 seedlings were procured, segregating in 548 green : 27 yellow-stained monoheterozygotes : 5 *lutescens* recessives. Field notations from this progeny show

that difficulties arose in definitively classifying the monoheterozygotes. We are therefore justified in counting together the green and yellow-stained plants for the analysis of probability. We thus have the found ratio 575 : 5 (the theoretical ratio for 63 : 1 would be 570,9 : 9,1). $\chi^2 = 1{,}877$ and $0{,}20 > P > 0{,}10$, i. e. a fair correspondence. 100 F_2 plants classified as »green» were subjected to sand-dish F_3 growing in the laboratory. For lack of space the detailed results are omitted here; altogether 8.144 germinated seedlings were counted, representing an average of 81,4 seedlings per dish. Among the 100 progenies in F_3 6 were 3 : 1-segregating, and were thus shown to be wrongly marked. The remainder, 94, consisted of 65 constant green, 9 tri- and 20 dimerically segregating progenies. Corresponding expected numbers would be 61,0 : 13,2 : 19,8. χ^2 amounts to 1,601 and $0{,}50 > P > 0{,}30$, i. e. a good correspondence. A tetramerical segregation would theoretically require 72,7 green + tetra- : 12,2 tri- : 9,1 dimerically segregating progenies; $\chi^2 = 14{,}711$ and $P < 0{,}001$. Thus, the F_2 951 r 31 is unquestionably 63 : 1-segregating. As no further progeny in F_2 was procured, we are so far unable to discard one of the two possible alternatives *A* and *B*: *I*. If *B*: *I* were the valid one, Orion II should have the constitution $l_s l_s\ L_p L_p\ L_n L_n$; in such a case, however, the practical breeding crosses, carried out between Orion II and the variety Stormogul II in the Wheat and Oats Dept. at Svalöf (F_1 No. *1939*—909; F_2 No. *1941*—923 r 5—11, 15—32, 36—49, 53—55) would have given a 63 : 1 *lutescens* segregation. Stormogul II must have the monofactorial constitution $L_s L_s\ l_p l_p\ l_n l_n$, as it originates (TORSSELL, 1933) from a cross Klock II × Stormogul, both with precisely this monofactorial constitution. Now, in the large F_2 from the cross Orion II × Stormogul II no *lutescens* segregation was observed; therefore Orion II cannot have the bifactorial constitution according to alternative *B*: *I*. Only alternative *A* then remains. Thus, Orion II carries *the three factors* L_s, L_p *and* L_n. This is in complete accordance with the fact that Orion II originates from Ligowo II × 0668 (from Merø), Ligowo II having also all three *L* factors, as shown above, p. 342.

Lutescens monoheterozygote × *Seger*. — Cross Nos. 375 and 376 from 1938 gave three fully normal-green F_1 plants, grown in 1939. In 1940 the three F_2 progenies on p. 353 (below) were procured.

Although no recessives were observed in r 19, eight yellow-stained monoheterozygotes were counted. According to a trimeric segregation of this F_2 progeny, we should expect the theoretical numbers 73,9 : 7,8 : 1,3. χ^2 then amounts to 1,322 and $0{,}70 > P > 0{,}50$, i. e. a good

Progeny number of F_3	Number of seeds sown	Number of germinated seeds	Found: green/*lutescens*	Expected: green/*lutescens*	P-values	Mode of segregation
1	162	149	all green	—	—	—
2	113	101	» »	—	—	—
3	72	63	60 : 3	59,1 : 3,9	$0{,}70 > P \geq 0{,}50$	15 : 1
4	90	88	83 : 5	82,5 : 5,5	$0{,}90 > P > 0{,}80$	15 : 1
5	77	65	all green	—	—	—
6	142	118	116 : 2	116,2 : 1,8	$0{,}90 > P > 0{,}80$	63 : 1
7	128	115	all green	—	—	—
8	186	176	169 : 7	165,0 : 11,0	$0{,}30 > P > 0{,}20$	15 : 1
9	5	5	all green	—	—	—
10	126	111	108 : 3	109,3 : 1,7	$0{,}50 > P > 0{,}30$	63 : 1
11	53	53	39 : 14	39,7 : 13,3	$0{,}90 > P > 0{,}80$	3 : 1 [1]
12	81	69	68 : 1	67,9 : 1,1	$0{,}95 > P > 0{,}90$	63 : 1
13	334	315	all green	—	—	—
14	147	139	» »	—	—	—
15	93	85	» »	—	—	—
16	31	29	» »	—	—	—
17	141	135	132 : 3	132,9 : 2,1	$0{,}70 > P > 0{,}50$	63 : 1
18	33	31	30 : 1	29,1 : 1,9	$0{,}70 > P > 0{,}50$	15 : 1
19	69	61	all green	—	—	—
20	60	60	57 : 3	56,2 : 3,8	$0{,}90 > P > 0{,}80$	15 : 1
21	71	69	all green	—	—	—
22	131	128	» »	—	—	—
23	32	32	» »	—	—	—
24	47	45	» »	—	—	—
25	255	227	212 : 15	212,8 : 14,2	$0{,}90 > P > 0{,}80$	15 : 1
26	24	23	all green	—	—	—
27	48	45	» »	—	—	—
28	172	157	144 : 13	147,2 : 9,8	$0{,}30 > P > 0{,}20$	15 : 1
29	13	12	all green	—	—	—
30	159	151	» »	—	—	—
31	149	140	» »	—	—	—
32	116	102	» »	—	—	—
33	47	45	» »	—	—	—
34	186	180	» »	—	—	—
35	114	105	103 : 2	103,4 : 1,6	$0{,}80 > P > 0{,}70$	63 : 1
36	128	120	all green	—	—	—
37	151	145	» »	—	—	—
38	68	62	» »	—	—	—
39	45	39	37 : 2	36,6 : 2,4	$0{,}80 > P > 0{,}70$	15 : 1
40	35	33	all green	—	—	—
41	42	40	» »	—	—	—
42	113	91	89 : 9	91,9 : 6,1	$0{,}30 > P > 0{,}20$	15 : 1
43	136	125	all green	—	—	—
44	25	18	16 : 2	16,9 : 1,1	$0{,}50 > P > 0{,}30$	15 : 1
45	280	270	258 : 12	253,1 : 16,9	$0{,}30 > P > 0{,}20$	15 : 1
46	186	175	all green	—	—	—
47	94	91	» »	—	—	—
48	142	135	» »	—	—	—
49	163	153	144 : 9	143,4 : 9,6	$0{,}90 > P > 0{,}80$	15 : 1
50	44	43	38 : 5	40,3 : 2,7	$0{,}20 > P > 0{,}10$	15 : 1
51	111	90	89 : 1	88,6 : 1,4	$0{,}70 > P > 0{,}50$	63 : 1
52	118	102	100 : 2	100,4 : 1,6	$0{,}80 > P > 0{,}70$	63 : 1
53	176	168	all green	—	—	—

Progeny number of F_3	Number of seeds sown	Number of germinated seeds	Found: green/*lutescens*	Expected: green/*lutescens*	P-values	Mode of segregation
54	254	243	all green	—	—	—
55	213	212	» »	—	—	—
56	102	100	» »	—	—	—
57	59	55	47 : 8	41,2 : 13,8	$0{,}10 > P > 0{,}05$	3 : 1 [1]
58	145	136	all green	—	—	—
59	135	115	» »	—	—	—
60	285	271	» »	—	—	—
61	45	40	» »	—	—	—
62	117	116	110 : 6	108,7 : 7,3	$0{,}70 > P > 0{,}50$	15 : 1
63	191	159	156 : 3	156,5 : 2,5	$0{,}80 > P > 0{,}70$	63 : 1
64	242	223	221 : 2	219,5 : 3,5	$0{,}50 > P > 0{,}30$	63 : 1
65	347	331	all green	—	—	—
66	96	91	» »	—	—	—
67	129	113	» »	—	—	—
68	125	120	» »	—	—	—
69	345	308	305 : 3	303,2 : 4,8	$0{,}50 > P > 0{,}30$	63 : 1
70	44	41	all green	—	—	—

correspondence. The 75 green seedlings yielded, when harvested, 70 ripe plants (the others being devastated by frit fly attacks) the seeds of which were grown as F_3 in sand dishes; the results tabulated above.

The F_3 progenies thus showed only two wrongly classified monoheterozygotes. Among the rest 44 progenies were constant green, 10 segregated tri- and 14 dimerically. The theoretical expectation requires the ratio 44,1 : 9,6 : 14,3, i. e. an unusually striking correspondence. A supposed tetrameric distribution would require the ratio 52,6 green + + tetra- : 8,8 tri- : 6,6 dimerically segregating progenies. χ^2 then amounts to 9,849 and $0{,}01 > P > 0{,}001$. Thus a tetrameric distribution is highly improbable. To establish alternative *A* as the only valid one, we revert to the two F_2 progenies r 17 and r 18. Together, they represent 142 seedlings, among which no recessives or yellow-stained monoheterozygotes were observed. For a supposed trimeric segregation we should expect per 142 seedlings the ratio 126,5 : 13,3 : 2,2. Observed numbers are 142 : 0 : 0. If the two last classes are reckoned together, we get $\chi^2 = 17{,}399$ and $P < 0{,}001$; a trimeric segregation is thus excluded. There

Number of F_2 progeny	Kernels sown	Seedlings procured, altogether	Green ones	Yellow-st. monoheteroz.	*Lutescens* recessives
1940—951—r 17	61	50	all	—	—
r 18	115	92	all	—	—
r 19	136	83	75	8	0

[1] The F_2 plant wrongly classified as normal-green.

are very small chances of any yellow-stained monoheterozygotes having been overlooked in these two progenies, as F_3 revealed only two such mistakes out of 70 F_2 plants. Let us, however, suppose that the 142 green F_2 seedlings from r 17 and r 18 had consisted of 138 really green and, say, 4 monoheterozygotic plants, the latter wrongly reckoned as green. χ^2 would then amount to 9,758 and $0{,}01 > P > 0{,}001$, i. e. still a very great improbability of a trimeric segregation. Finally, since only the alternatives *A* and *B*: *I* are left to choose between, *B*: *I* can be definitely discarded, as the practical breeding cross Seger × Klock II gives no *lutescens* recessives. This would have been the case if Seger had had the constitution $l_s l_s\, L_p L_p\, L_n L_n$ of alternative *B*: *I*. Thus, Seger has *the three L factors* L_s, L_p *and* L_n.

3. SOME GENE-PHYSIOLOGICAL OBSERVATIONS ON THE IDENTITY OF THE THREE L FACTORS.

In the foregoing we have at intervals met with the fact that yellow-stained monoheterozygotes have been wrongly classified as green plants. In certain years, however, a practically faultless classification was possible, especially when dry periods coincided with the 3—4-leaf seedling stage. It should be pointed out that the yellow stains or dots on the leaves are typically recognizable only during 8—14 days. They then vanish rapidly, and the seedlings are quite indistinguishable from normal-green ones. The point of onset of the yellow-staining on the leaves is more marked and easier to observe than is the »vanishing point» of the yellow dots; both observations, however, require considerable experience.

It is appropriate to revert to ÅKERMAN's above-cited work of 1929 and recall his hypothesis on the *L* factor action upon chlorophyll development. ÅKERMAN (l. c., p. 596), following the theories of GOLDSCHMIDT (1927), assumes that in a yellow-stained monoheterozygote a factor L_1 exists for normal green colour together with its allelomorph l_1, the latter causing the development, characteristic of the *lutescens* plants. Both genes cause a series of reactions: L_1 in the direction of chlorophyll synthezis, l_1 in the direction of chlorophyll destruction. The two series of reactions proceed at different rates, illustrated by the curves in Fig. 1. At the outset the first one goes faster, the seedlings thus showing normal-green colour. After some time (point t_1 in the diagram) the l_1 curve intersects that of the L_1 curve: for a short period the chlorophyll-destroying reactions overweigh; hence the appearance of the yellow dots or stains on the leaves. After a short time, however, the chlorophyll-

synthesizing reactions regain the upper hand: curves L_1 and l_1 once more intersect (t_2 in the diagram). All leaves, developed from now on, are normal-green; the earlier yellow-stained ones regain almost entirely their normal-green colour. (Later experiences have shown that the first and second leaf may, under certain circumstances, keep the yellow dots up to withering — the dotted character then having been so pronounced

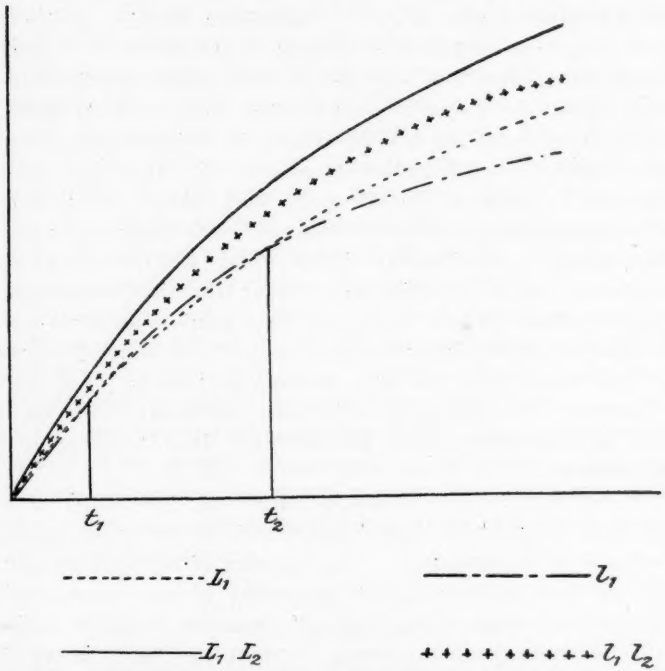

Fig. 1. Reaction mechanism of chlorophyll building and destruction, caused by L and l factors in hexaploid oats. (Reproduced from ÅKERMAN, 1929, p. 597.)

that the further ontogenesis of the said leaves is too short to let the change in dominance appear.) In the leaves of bi- and trifactorial heterozygotes (with ÅKERMAN's nominations 1929 $L_1l_1\,L_2l_2$ and $L_1l_1\,L_2l_2\,L_3l_3$) no yellow-staining appears, which is explained by the assumption that the rate of the synthesizing reactions is increased in the presence of two or three L factors in a greater degree than the rate of the destructive reactions. Hence the diagram shows that the curves for L_1L_2 and l_1l_2 do not intersect.

The hypothesis thus put forward introduces some further aspects of the *L* factor action. In the writer's large field F_2 progenies of *lutescens* segregating plots it was observed from the outset that — with small variations — all monoheterozygotes developed their yellow dots almost simultaneously, *quite independently of the different genic environment from which the segregation was procured.* It is true that at the seedling stage no great differences in development can be reasonably supposed to exist even in an F_2 from so widely differing strains as the *lutescens* monoheterozygote used (product from Klock II × Abeds Nova and back-cross) and, e. g., extremely early north-Scandinavian oats (such as Thorshavre; results not recorded above). Nevertheless, the monoheterozygote dots began to develop in the F_2 *lutescens* monoheterozygote × Thorshavre almost simultaneously with the F_2 of the same heterozygote × Ligowo II (observations from 1944). *No real differences between different monoheterozygotes in the same di- or trimerically segregating F_2 could be established in respect to the time of yellow-staining onset.* Since in a fairly large di- or trimerically segregating F_2 progeny from these crosses the two or three possible monoheterozygote types must necessarily occur together, the primary supposition was, of course, *that the action of the three allelomorph pairs $L_s l_s$, $L_p l_p$ and $L_n l_n$ was identical* — in complete accordance, naturally, with the reduplication hypothesis regarding the origin of these homologous, polymeric *L* factors.

In a systematic work on this problem, the three different monoheterozygotes should have been isolated and directly tested together in the field and in the laboratory. The question of factorial identity was, instead, attacked by studying the appearance of the yellow-spotting in some trimerically segregating F_2 and F_3 progenies available during the years. The onset of yellow-spotting (= point t_1 in Fig. 1) is rather easy to determine in favourable years. The vanishing of the dots (re-greening) is, on the other hand, as a rule too prolonged to permit any definite determination. The results from three favourable years all point in the direction that it is impossible to establish any definite difference in respect to the t_1 point between the three different monoheterozygotes. The 28 monoheterozygotes classified in plot 1941—952 r 40 (F_2 from the cross *lutescens* monoheterozygote × Ligowo II; see p. 342) were carefully followed day by day after sprouting: $^{14}/_{6}$ 2 heterozygotes showed dots, $^{15}/_{6}$ 25 were in all counted, $^{16}/_{6}$ 28 in all, $^{17}/_{6}$ 28 in all, and, finally, $^{18}/_{6}$ still 28 in all. Thus, the dry, sunny summer of 1941 in this F_2 caused the yellow dots to appear *practically on the same day,* $^{15}/_{6}$. It was noted

in the field-books that the yellow-stained plants had all but 2 re-greened on the 30th of June, the two plants still showing dots on the 1st and 2nd leaf, for the reasons mentioned above (p. 355). In other years, e. g. 1944, the onset of yellow-spotting is prolonged for up to 5—6 days; the distribution of the t_1 determinations on the various monoheterozygotes is, however, very uniform. The 28 monoheterozygotes from above gave 24 full-grown F_3 plants; they were harvested and grown in 1944 in the field. All 24 segregated 3 : 1; the onset of yellow-staining occurred rather uniformly $^{20-25}/_5$ in the 24 progenies.

Studies have also been made on the phenotypical variation in the isolated monoheterozygote $L_s l_s\, l_p l_p\, l_n l_n$, used in all crosses, on the one hand, and the variation in the population of the three monoheterozygotes — side by side in the field —, on the other. The experience thus gained naturally does not give any definite proof of the *L* factor identity, but the observations *strongly indicate such an identity as probable.* Returning once more to the diagram in Fig. 1, it is difficult to understand why a complicated reaction mechanism, letting a certain well-defined chlorophyll defect develop, reach a maximum and vanish, should act in such a consistent way if it were not conditioned by factors of complete — or almost complete — identity. The *l* factors obviously have the capacity of interfering so decisively with the normal reaction chain of chlorophyll development that their action seems practically independent of the genetic environment, as far as the crosses studied have shown. The *lutescens* case in hexaploid oats will thus serve as *the most cogent argument hitherto adduced for factorial reduplication identity caused by polymery.*

4. DISCUSSION.

In Fig. 2 the results hitherto gained (in all 15 varieties analysed) are tabulated in a scheme of evolution comprising varieties with three-, two- and monofactorial constitution in respect of *L* factors. Just as in the *chlorina* case, it is possible to trace phenotypically hidden mutational steps from the phylogenetically primary three-factorial set $L_s L_s\, L_p L_p\, L_n L_n$ down to the monofactorial $L_s L_s\, l_p l_p\, l_n l_n$ stage; the two other monofactorial stages possible have not yet been found, owing to the limited number of varieties tested. In Fig. 2 dotted arrows indicate that the corresponding varieties may belong to different alternatives, the choice between which must at present be left undecided. Unbroken arrows indicate the possible evolutionary directions of the

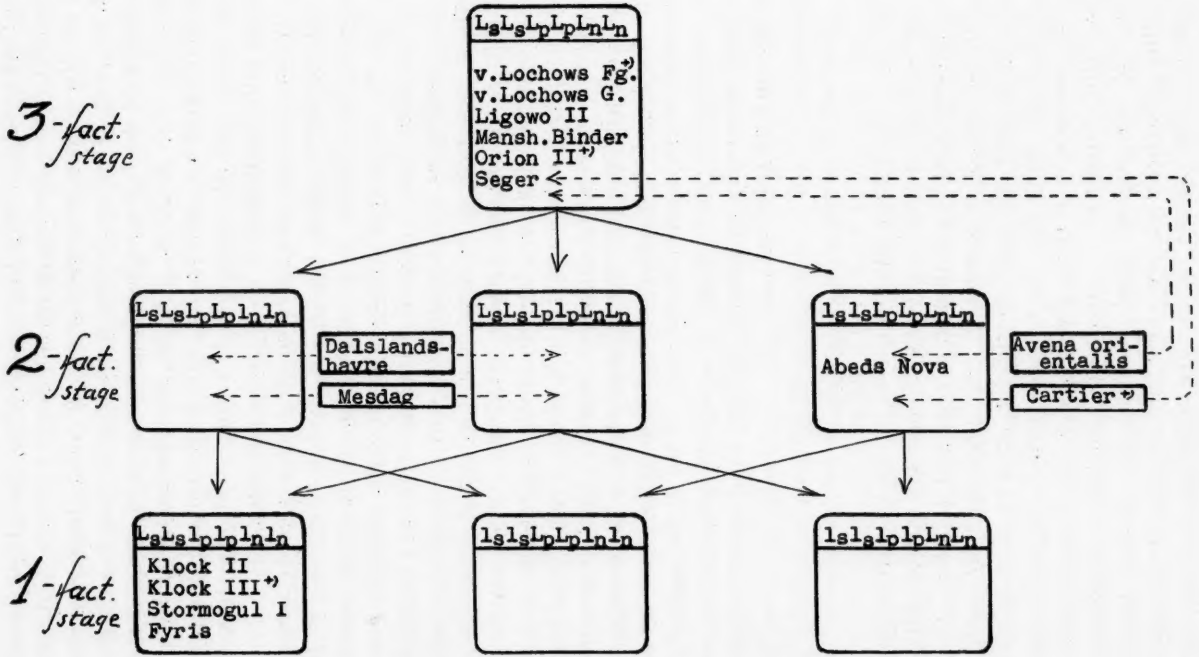

Fig. 2. Sets of *lutescens* factors in various hexaploid oat varieties. *) Cross-bred strains.

lutescens factorial sets; the phylogenetic development of the tabulated varieties has, of course, nothing to do with these unbroken arrows.

We thus again meet with the same phenomenon so clearly shown in regard to the above-mentioned *Chlor* factorial sets of hexaploid oats. No monofactorial *lutescens* strain has, so far, produced *lutescens* plants by obvious mutation. Nevertheless, the same conclusions drawn in the *chlorina* case must be repeated here. Thus, oats of the same ecologically well-defined groups show the same factorial set. The pure Probsteier lines Mansholt's Binder and Seger are all trifactorial, together with the probably closely related Ligowo II; only Abeds Nova has among the Probsteier types advanced one step towards fewer *L* factor couples. The central-Swedish black-hulled oats Klock II, Stormogul I and Fyris all show the monofactorial $L_sL_s\, l_pl_p\, l_nl_n$ stage — this in sharp contrast to the situation as regards *Chlor* factor sets (Ch. II). It should be pointed out that both in the case of *chlorina* and *lutescens* the cross-bred strains tested in this cross analysis naturally offer only limited interest; they have been included chiefly for practical considerations. From the practical cross breeding work many additional, rather definite conclusions can be drawn: e. g., the variety Klock III, tested in the experiments, originates from the cross Klock II × Stormogul I, which yielded no less than four marketed black-hulled central-Swedish oat strains: Klock III (tested), Engelbrekt, Engelbrekt II and Stormogul II. The latter three varieties must then reasonably also have the *lutescens* set $L_sL_s\, l_pl_p\, l_nl_n$.

In spite of the limited number of varieties that could be tested in view of the extensive work needed for each variety, the writer managed to reveal at least two varieties (line 29/100 from Dalslandshavre, Mesdagshavre), both of which must carry an *L* factor set with two *L* couples but definitely different from the Abeds Nova *L* factor set, a second transitional stage between the three- and monofactorial constitution thus being established. Hence, 4 (or perhaps 5?) of the possible 7 factorial distributions have been found in reality to exist which — together with the analogous circumstances in the *chlorina* case — strongly supports the validity of the view regarding progressive mutation.

The frequency of the *L* factor mutations must be extremely minute, since (a) no mutations to L_sl_s have been observed in the monofactorial market strains with the constitution $L_sL_s\, l_pl_p\, l_nl_n$ during so many years of growing and controlling at Svalöf with substations — and foreign breeding stations as well —, and as (b) trifactorial strains still exist.

IV. THREE NEW SPONTANEOUS OAT CHLOROPHYLL MUTATIONS, FF. ALBOVIRESCENS, LUTEOMACULATA AND TIGRINA-1 (BRIEF REPORTS).

Owing to lack of space a more detailed account has to be postponed to a later volume of this journal. Below, the chief features of the new mutations will, however, be recorded in brief.

The f. *albovirescens* was found by the writer in an F_5 from a cross between the two hexaploid cross-bred oat lines 01422 b_2 and 01431 b. Investigations on the F_1—F_4 generations made it clear that the *albovirescens* segregation in F_5 must be ascribed to spontaneous mutation and not to mere segregation of differently distributed polymeric factors (cf. the discovery of the f. *lutescens* by ÅKERMAN, 1922). Regularly monomeric segregations were obtained; it is not yet clear if polymeric inheritance exists for this new chlorophyll mutation, owing to the extreme difficulties of crossing the semilethal-subvital recessive *av av* with the parent lines.

The recessive entirely coincides with GUSTAFSSON's subgroup *viridoalbina* and would have been designated according to GUSTAFSSON but for its discovery in 1939 before his system of 1940 was published. The recessives are greatly retarded in development; only 50 % reach maturity as small dwarfs, setting 2—5 seeds. Each leaf is from the beginning pure white except for the leaf-tip which is very slightly yellowish-green (CDC = 296)[1]. In later stages the tip greens more and more, and finally the whole leaf gets green, never, however, attaining the full-green of the normal neighbour plants *Av av* or *Av Av* (CDC = 311—312 for final *albovirescens* leaf stages; 326—327 for the normal leaves of the same order).

The f. *luteomaculata* was discovered by ÅKERMAN in 1937 (unpublished) in the hexaploid oat Guldregn II (from Seger × Guldregn I; ÅKERMAN, 1928) and was handed over to the writer for closer studies. Selection- and cross-experiments in 1937—1941 proved the new aberration to be non-Mendelian, exclusively maternally inherited. In my opinion, the inheritance of the f. *luteomaculata* could be interpreted according to the classical scheme of CORRENS (1908) for his *albomaculata* type, afterwards discussed by GREGORY (1915), WINGE (1919) and CORRENS (1931). The works of RENNER (1934, 1936) on the genetic autonomy of the plastids give good reason to assume a plastid mutation as causing the appearance of the f. *luteomaculata*. The exclusively maternal inheritance manifests itself regularly also in reciprocal crosses with the f. *chlorina*.

The aberration is fully vital and characterized by irregularly distributed yellow or yellow-green spots (CDC = 281—283) on the second and third seedling leaves; the normal-green colour of these leaves corresponds at this stage of development to CDC = 301—303. Later leaves — as also in general the first leaf — do not exhibit any such spots. In these spots the mesophyll cells show chloroplasts in a state of dissolution: all transitional stages from normal to wholly decomposed plastids are observed. Cells even with totally degenerated plastids do not perceptibly lose their turgor. Therefore, in the yellow spots we never find any »shrunk» features of the leaf — this in contrast, e. g., to the behaviour of the members of the *tigrina* group in barley and oats and the »zebra» forms in maize. The *tigrinas* show a more advanced necrosis, the degenerating cells losing their turgor and being squeezed together with transverse leaf area deformations as the result.

RANDOLPH (1922) and ZIRKLE (1929) have discussed partly similar plastid degeneration features in maize as were found in this f. *luteomaculata*.

The f. *tigrina* was found by ÅKERMAN in 1935 in a line of the diploid *A. strigosa* from Hinneryd, Småland, as a spontaneous mutation. In my experiments this form has shown a regular monomeric segregation, also when crossed to other *strigosa* varieties: *A. strigosa* v. *glabrescens* MARQUAND (THELL.) and *A. strigosa* ssp. *pilosa* v. *alba* MARQUAND (cf. MARQUAND, 1922, and JONES, 1931, as to taxonomy; both

[1] »CDC» used as an abbreviation for »Code des Couleurs» by KLINCKSIECK et VALETTE, 1908.

varieties from Wales). The recessive is semivital, sets a few seeds and holds an intermediate position in vitality between the lethal induced barley *tigrinas* (cf. GUSTAFSSON, 1940) and the only slightly subvital »zebra» types in maize (DEMEREC, 1921, 1924; STROMAN, 1924; HAYES, 1932). Solitary *tigrina-X* mutations have been produced from both X-rayed diploid wheats (L. SMITH, 1939, »yellow-zoned», p. 18), diploid and hexaploid oats (Ch. V). The wheat mutation is quite vital; the diploid oat mutations (*tigrina-X-1* in *A. strigosa* and *tigrina-X-1* in *A. brevis*) are fully vital and lethal respectively. The two hexaploid oat *tigrina-X* mutations found are lethal—sublethal. No spontaneous *tigrina* has as yet been found in barley.

The anatomical study of the *tigrina* recessives reveals an advanced necrosis of chloroplasts and mesophyll cells not hitherto found in any oat or wheat chlorophyll aberration. The chloroplast destruction advances to an end-result of diffuse, clumped, strongly anthocyanine coloured masses in whole complexes of mesophyll cells. These cell-complexes partially or wholly lose their turgor and are pressed together by the surrounding normal cells, resulting in sunken, zonated longitudinal stripes of the leaf and a transverse contraction of the leaf area over the zones. These are developed only on the first and second seedling leaf. Leaves of higher order do but exceptionally show such a zonation. The development of the recessive is much retarded, and few seeds produced.

V. INDUCTION OF X-RAY CHLOROPHYLL MUTATIONS IN DI-, TETRA- AND HEXAPLOID OATS.

1. TECHNIQUE AND MATERIAL.

All experiments recorded in Chapters V and VI have been carried out with X-rayings of dry seeds. Firstly, preliminary experiments with greenhouse cultures were undertaken, which are reported in FRÖIER's paper (1941) on the germination and sprouting ability of oats and wheat after different X-ray dosages, and in FRÖIER and GUSTAFSSON's (1941), where experiments with barley are also recorded. Generally, the doses applied in these experiments were rather high, ranging to 60.000 r (intern. X-ray units), at which dose, however, the sprouting of the hexaploid variety Seger oats (water-content at radiation 13 %; intensity 2.500 r/minute) was totally inhibited. If the water-content is higher, e. g., 16—20 %, the X-raying will cause such heavy effects that even hexaploid wheat does not sprout at doses higher than 25.000 r. A comprehensive report on the results of these experiments will be published by FRÖIER and GUSTAFSSON before long; hence only the main results will be briefly recorded here when necessary for the discussion.

It is very obvious that the tetra- and hexaploid oats are much more resistant to X-ray treatment than are the diploids; Table 4 gives the percentage of seedlings obtained in field-grown X_1 progenies from various oats and dosages. Germination, vigour and fertility of the X_1 in the polyploid species of the genus is decidedly better than in the diploids on account of gene and chromosome duplication. Even deleterious changes induced at a certain locus in one chromosome will as a rule be counterbalanced in the polyploids by a normal, non-affected condition in the other homologous chromosomes. The first direct and still most convincing proof of greater X-ray resistance following gene and chromosome duplication was the comparison autotetraploid Opal B barley *versus* its diploid mother line, made by MÜNTZING (1941, 1942). A striking comparison of the different reaction to X-rays of a diploid and a hexaploid at about the same water-content is found in FRÖIER and GUSTAFSSON (1941) in Figs. C and D, pp. 50—51, where Seger oats and Maja barley are compared. Disregarding the not very important difference in water-content (0,8 %) between the two species, we find a strikingly pronounced resistance of the hexaploid oat strain in the interval 20.000—50.000 r as compared with the barley diploid. The seedlings were grown in soil-boxes in the greenhouse.

In field growings, however, the mortality of X-rayed seeds is considerably increased. The sowing with the marking-boards places each kernel at 5 cm depth in the soil, the consistence of which is less favourable than in the greenhouse experi-

TABLE 4. *Percentage of seedlings counted in X_1 from X-rayed di-, tetra- and hexaploid oats. X_1 grown in the field.*

(Numbers in brackets = kernels sown.)

Field number	Species, variety	Water-content, %	Seed provenience	Untreated control, % seedlings germin.	Percentage of seedlings germinated				
					5.000 r	10.000 r	15.000 r	20.000 r	30.000 r
	Diploids:								
1939—959 —r 2 + r 8	*A. strigosa* SCHREB.	12,6	1938	—	11,9 (2.000)	—	—	—	—
1939—952 —r 21—30	» (same line as above)	12,6	1938	85,4 (1744)	—	—	—	—	—
1940—959 —r 1	» »	12,1	1939	—	46,8 (600)	0,0 (600)	0,0 (600)	0,0 (600)	—
1940—952 —r 11—15	» »	12,1	1939	87,8 (801)	—	—	—	—	—
1941—951 —r 46—50	» »	12,3	1940	54,4 (90)	68,8 (500)	7,6 (500)	0,0 (500)	0,0 (1.000)	—
1940—959 —r 2	*A. brevis* THELL.	10,8	1936	no contr.	44,8 (600)	0,0 (600)	—	—	—
1941—951 —r 43—45	» (same line as above)	11,1	1936	36,7 (60)	5,3 (600)	0,2 (600)	—	—	—
	Tetraploids:								
1941—951 —r 34—35	*A. barbata* (POTT) THELL.	12,0	1937	12,0 (50)	10,8 (454)	—	—	—	—
1941—951 —r 36—38	» (same line as above)	12,6	1940	44,0 (50)	62,7 (102)	38,6 (101)	—	—	—
1943—963 —r 1—5	*A. abyssinica* (HOCHST.) THELL.	10,2	1942	69,0 (100)	72,0 (500)	39,2 (500)	0,0 (500)	0,0 (1.000)	—
	Hexaploids:								
1941—951 r 1—4	Klock II	12,1	1940	88,2 (90)	—	70,2 (500)	—	11,6 (500)	0,2 (1.000)
r 5—r 8	Stormogul I	12,5	1940	76,7 (90)	—	76,8 (500)	—	9,4 (500)	0,8 (1.000)
r 9—r 12	Engelbrekt II	13,0	1940	82,2 (90)	—	63,0 (500)	—	2,0 (500)	0,3 (1.000)
r 13—r 16	Guldregn I	12,2	1940	87,8 (90)	—	67,4 (500)	—	0,6 (500)	0,4 (1.000)
r 17—r 20	Abeds Nova	13,1	1940	76,7 (90)	—	15,0 (500)	—	0,6 (500)	0,0 (1.000)
r 21—r 24	Seger	13,2	1940	82,2 (90)	—	36,8 (500)	—	0,4 (500)	0,0 (1.000)
1943—963 —r 6—r 9	»	10,4	1942	76,0 (100)	—	67,6 (500)	—	53,4 (500)	2,6 (1.000)
1941—951 —r 25—r 27	Sapeli	11,9	1938	71,0 (100)	78,4 (250)	52,4 (250)	—	—	—
1941—951 —r 28—r 31	*A. fatua* L.	11,6	1939	51,1 (90)	—	7,0 (300)	—	0,0 (300)	0,0 (300)
r 32—r 33	*A. sterilis* (origin: cf. p. 314)	11,3	1936	48,3 (60)	50,3 (300)	—	—	—	—

ments, where the soil was sifted to optimal particle size. The conditions of temperature and humidity are likewise far less favourable in the field than in the greenhouse. Hence, the possibilities of getting mature field-grown plants from seeds treated with higher X-ray dosages are rather restricted. Up to now no hexaploid oat (or wheat) X_1 generations have been obtained in the writer's (and GUSTAFSSON's) experiments from dosages above 30.000 r. This is in good accordance with the findings of FRÖIER, GELIN and GUSTAFSSON (1941), who clearly demonstrated that even dosages of 20.000 and 25.000 r caused distinct chromosome disturbances of practically 100 % of the somatic cells studied. Cf. also the report of L. SMITH (1943) on the same subject.

In Table 4 the various field-grown X_1 oat generations from 1939—1943 are given together with percentages of seedlings germinated, numbers of sown kernels, water-content and provenience of irradiated seeds.

The pure line of *A. strigosa* used in these experiments is the same as was selected from the Hinneryd *strigosa* population (cf. p. 360). The material of *A. brevis* was procured from Aberystwyth by courtesy of the Deputy Director, Dr. R. O. WHYTE. Among the tetraploids the *A. barbata* originates from Prof. Dr. E. SCHIEMANN, Berlin. The *A. abyssinica* line was obtained from the late Dr. FREISLEBEN of Halle. As to the hexaploids, the Sapeli oats originate from Finland, representing a pure line from Finnish land oats. The *A. fatua* pedigree originates from Värmland (pedigree 1917).

In Table 4 the number of mature plants with panicles for each dosage has not been tabulated, nor have the percentages of plant sterility, where such have been determined (cf. p. 371 and later). In the following such data will be found in connection with the reports on chlorophyll mutations in the various X_2 generations from the oats investigated. It should be stressed that the comparisons in Table 4 (and consequently also between the mutation frequencies obtained in the different X_2 generations) are not — as easily seen — strictly adequate, since (a) the water-content of the irradiated seeds is varying, (b) the provenience of the irradiated kernels is not uniform, and (c) the number of untreated control kernels and of irradiated kernels is varying. For several reasons, however, the comparisons must be recognized as valid enough to furnish us with at least the primary aspects of induced oat chlorophyll mutation.

The sterility induced in the X_1 generation must to a great extent be attributed to intra- and inter-chromosomal alterations, the number of which can be supposed to display a rather distinct parallelism with the mutation frequencies found. Among others, GELIN (1941) has shown a marked parallelism in irradiated barley between the percentages of disturbed cells, sterility and mutation rate. Hence a determination — as strict as possible — of the X_1 plant-sterility is desirable. The total sterility of an individual X_1 plant (i. e. the number of empty flowers divided by the total number of flowers) probably rather adequately denotes the cytological status of the plant in question. In barley such a sterility determination is much easier to carry out than in oats or wheat, where the true number of empty flowers could hardly ever be satisfactorily counted. Especially oat panicles vary greatly in spikelet and flower number, owing to a wide modificability of development even in untreated plants. For this reason sterility analyses were made only in certain selected X_1 generations considered as successfully X-rayed and at the same time as homogeneously and normally developed series. Examples of such sterility analyses are found on p. 371 and later.

In the following the chlorophyll mutations induced in the various treatment series are recorded in numerical order for each species. Unless otherwise stated the intensity of the X-raying has been from 2.200 to 2.500 r/min (focal distance 7 cm; 4 mA; 175 kV; Cu-filter 0,05 mm + 1,00 mm carton). In certain earlier series, however, the intensity was not higher than 72 r/min. Since according to TIMOFÉEFF-RESSOVSKY (1937) a straight-line proportionality exists between X-ray quantity and induced mutation frequency, the differences in intensity thus mentioned cannot be supposed to disturb the essential comparisons between the series.

As to the nomenclature of the mutations, GUSTAFSSON's system (1940) is used. The abbreviation »CDC» is used for references to »Code des Couleurs» (see p. 360).

2. INDUCED CHLOROPHYLL MUTATIONS: DIPLOID OATS.

In 1939 the Hinneryd line of *A. strigosa* was irradiated with an intensity of 72 r/min; dosage 5.000 r; water-content 12,6 %. 1.000 kernels were irradiated; they gave an X_1 of 173 seedlings and at harvest 152 panicle-carrying plants. Separate panicle-progenies were not grown, but it was noted that the 152 plants comprised 243 panicle-carrying tillers, fertile or partly fertile. Completely sterile panicles were discarded; hence no attempt at a closer sterility analysis was made in this X_1 generation (cf. p. 371). The X_2 from these 152 plants was grown in 1940. The chlorophyll mutations obtained are recorded below; on account of space limits only the essential characteristics can be mentioned.

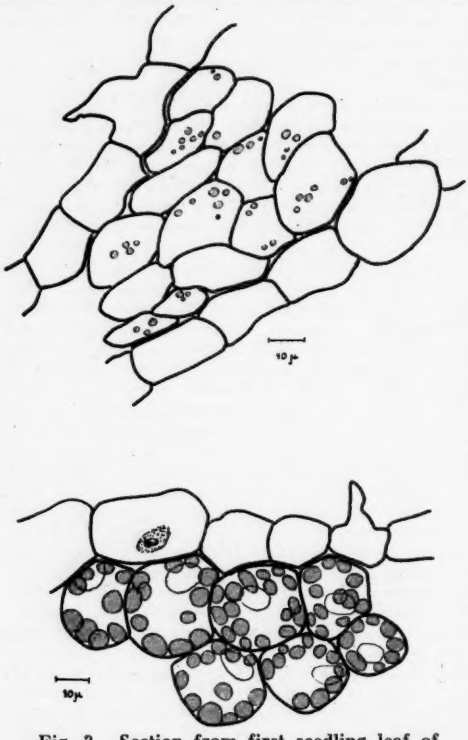

Fig. 3. Section from first seedling leaf of *albina-X-1* and normal-green *A. strigosa* (below).

Albina-X-1. — A well-defined, extremely rapidly withering type. Only the first leaf develops — to 5 cm length; the seedling then dies. Segregation in X_2 (1941) regularly monomeric: in the field 51 *albina-X-1* to 198 normal-green against expected 62,3 : 186,7 with $0{,}10 > P > 0{,}05$; in the greenhouse in sand dishes 106 *albina-X-1* to 288 normal-green against expected 98,5 : 295,5 with $0{,}50 > P > 0{,}30$. Chloroplasts in the recessives very small and varying in size, *among them, however, some faintly but distinctly green ones.* Most chloroplasts are, however, devoid of chlorophyll and carotinoids. Fig. 3 shows sections from *albina-X-1* and normal *strigosa* leaf from seedlings of conformal development (5 cm); section made $^1/_2$ cm from leaf tip. Original magnification when drawing = 1.090 ×. Hence RANDOLPH's statement of 1922 that *albina* mutations also contain plastids, although the plastid development is permanently retarded, is supported very beautifully. The criticism by VON LOUI (1931) against RANDOLPH's results can hardly be applied to the present writer's obvious *albina-X-1* case. The average chloroplast size (largest diameter) of normal-green *strigosa* = $5{,}1 \pm 0{,}12\,\mu$ (80 chloroplasts counted) and of *albina-X-1* = $2{,}1 \pm 0{,}09\,\mu$ (41 chloroplasts counted).

[*Technique of chloroplast studies.* — Razor-cuttings, 50—60 μ in thickness have been made of the living material, the sections immediately being plunged into a 1 $^1/_2$—2 mol cane-sugar solution and then rapidly put under the microscope. The drawings were made with a camera lucida, magnification about 1.050 ×. During the 5 minutes necessary for adjustments and drawing, no change in the dimensions of plastids attributable to plasmolytic effects could be observed. GUSTAFSSON (1942) adopted the same technique in plastid studies on barley mutations. His paper contains a critical review of earlier, rather scarce, chloroplast studies on chlorophyll mutations with exhaustive literature references. The experiences of the present writer fully coincide with those of GUSTAFSSON in regard to the use of strong fixatives. These fluids almost always produce artefacts, so that no reliable comparison, e. g., between aberrant and normal-green form can be undertaken (cf. GUSTAFSSON, l. c., p. 485, in his criticism of BERGMAN's figures in EULER, BERGMAN, HELLSTRÖM and BURSTRÖM, 1936).]

Albina-X-2. — The first seedling leaf develops to a length of about 8 cm; the seedling persists for about 5—6 days and then withers off (cf. the very rapid withering of *albina-X-1* above). Mode of segregation not yet revealed. Most probably a pronounced deficit of recessives: the X_2 progeny 1940—958—95 showed only 5 *albina-X-2* to 67 green. Repetitions of the segregation in X_3 and X_4 have not yielded any conclusive results on account of the too small number of green X_2 plants selected. The chloroplast development is strongly restricted: no traces of green plastids (proplastids) could be seen in sections of the leaves.

Albina-X-3. — The X_2 progeny 1940—958—122 segregated into 2 *albina-X-3* and 6 normal-green seedlings. The latter gave only 3 seed-carrying plants and did not repeat the segregation. The *albina-X-3* was primarily called »cochleo-albina» because of its peculiarly spirally twisted first seedling leaf. Mode of segregation unknown, perhaps monomeric. Seedling persists 5—8 days (or more?). One of the seedlings was studied as to chloroplast development: the sections showed only small proplastids or somewhat larger colourless ellipsoidic corpuscles.

Viridoalbina-X-1. — The X_2 progeny 1940—958—179 segregated into 3 *viridoalbina-X-1* and 40 normal-green seedlings. The recessive has the leaf base white with lilac margins, tip faintly yellow-green (CDC = 277). Withers about 5 days old at first-leaf stage. Monomerically inherited. No indications of gametic or zygotic lethality found. Below, the segregation in X_3 from arbitrarily chosen X_2 plants of normal-green colour is given:

Field number 1941	Field number 1940	Found: *viridoalbina-X-1*/normal-green	Expected: *viridoalbina-X-1*/normal-green (monomeric ratio)	P-values (monomeric ratio)	Number of kernels sown	Germinated seeds, %
960—	958—					
103	179	45 : 131	44 : 132	$0{,}90 > P > 0{,}80$	225	78,2
104	»	— : 100	—	—	147	68,0
105	»	21 : 98	29,8 : 89,2	$0{,}10 > P > 0{,}05$	171	69,6
106	»	79 : 215	73,5 : 220,5	$0{,}50 > P > 0{,}30$	396	74,2
107	»	39 : 153	48 : 144	$0{,}20 > P > 0{,}10$	288	66,7
108	»	36 : 134	42,5 : 127,5	$0{,}30 > P > 0{,}20$	210	81,0
109	»	56 : 189	61,3 : 183,7	$0{,}50 > P > 0{,}30$	354	69,2
110	»	— : 109	—	—	153	71,2
111	»	— : 90	—	—	189	47,6
112	»	35 : 126	40,3 : 120,7	$0{,}50 > P > 0{,}30$	219	73,5

Viridoalbina-X-2. — More viable than the preceding type. Base pure white, tip green (CDC = 301). Viable up to three-leaf stage, then rapidly withers off. Possibly monomerically inherited: in the X_2 1940 two *viridoalbina-X-2* were counted together

with 52 normal-green seedlings. Among 15 selected green plants only one gave a segregating X_3 progeny, consisting of 23 recessives : 100 normal-green. Expected numbers for a 3 : 1-ratio would have been 30,8 : 92,2 with $0{,}02 > P > 0{,}10$, i. e. a fair agreement for a monomeric ratio.

Lutescens-X-1. — The X_2 progeny 1940—958—92 segregated into 3 *lutescens-X-1* and 2 normal-green; the green plants did not repeat the segregation in X_3. A distinct *lutescens* type: seedling withers on first-leaf stage, beginning at the tip and progressing basewards. Withered seedling yellow-white just as the *lutescens* known in hexaploid oats. Inheritance unknown.

Lutescens-X-2. — The X_2 progeny 1940—958—136 segregated into 5 recessives and 30 normal-green seedlings. In X_3 five non-segregating and one 3 : 1-segregating progenies were obtained, the latter giving 72 *lutescens-X-2* to 194 normal-green. Expected numbers would be 66,5 : 199,5 with $0{,}50 > P > 0{,}30$. The *lutescens-X-2* recessive reaches the 2- (perhaps 3-) leaf stage but already begins to show decomposition of

Fig. 4. Chloroplasts from mesophyll cells of first leaf of normal-green *A. strigosa* (upper picture) and of the same leaf of *lutescens-X-3* (lower picture).

chlorophyll at an early 2-leaf stage. Withers off, the leaves having been yellow during the previous three or four days.

Lutescens-X-3. — The X_2 progeny 1940—958—158 segregated in 1 *lutescens-X-3* : 20 normal-green. This type withers at first-leaf stage. In contrast to *lutescens-X-1* this withering process advances rather slowly, as subsequent X_3 growings have proved. The mode of inheritance significantly deviates from at least a 3 : 1 and even a 15 : 1 Mendelian ratio. Thus, among 13 X_3-progenies 6 segregated in total 1.257 green : 23 *lutescens-X-3*, i. e. only 1,8 % recessives. The study of this interesting form is still in progress. All segregated recessives are absolutely morphologically uniform. Unequal distribution of chromosomes carrying mutated genes (cf. PHILP, 1935) might be responsible for the segregation in question. A closer cytogenetic study will perhaps reveal the causes of the aberrating numbers. The chloroplasts of the *lutescens-X-3* recessives have been studied. *Their maximum size* — in the deepest green parts of the first leaf (CDC = 301) — *is distinctly smaller than in normal-green leaves*; cf. Fig. 4.

The *lutescens-X-3* chloroplasts in Fig. 4 measure a maximum diameter of $2{,}5 \pm 0{,}045\ \mu$; those of normal-green leaves $4{,}2 \pm 0{,}073\ \mu$. Altogether 83 chloroplasts of the *lutescens-X-3* and 74 of the normal-green leaf were measured. The chloroplasts

of the mutation *lutescens-X-3* hence only reach on an average 60 % of the normal diameter before the seedling dies.

Lutescens-X-4. — In the X_3 progeny 1940—958—163 4 *lutescens-X-4* recessives were counted together with 15 normal-green. About 50 % of the green plants repeated the segregation in X_3; regular monomeric ratios were obtained. No indications of gametic or zygotic lethality were found. The recessives die off at 1- or 2-leaf stage. They are distinctly different from the other *lutescens* types found. Emergence of the first leaf is perceptibly retarded. The 2nd leaf also emerges slowly, compared with normal leaves.

Lutescens-X-5. — This type was first called »strigoso-lutescens» on account of its striking similarity to the well-known f. *lutescens* in hexaploid oats (ÅKERMAN, 1922). The heterozygote has, however, normal-green colour during its whole development — in contrast to the hexaploid *lutescens* monoheterozygote. The feature of withering and dying off is practically identical. Inheritance regularly monomeric. No signs of zygotic lethality yet found. In the tabulation below the segregation of *lutescens-X-5* in X_3 field-grown progenies from arbitrarily chosen green X_2 plants is illustrated:

Field number 1941	Field number 1940	Found: *lutescens-X-5*/ normal-green	Expected: *lutescens-X-5*/ normal-green	P-values (monomeric ratio)	Number of kernels sown	Germinated seeds %
960—	958—160					
80	1	—: 199	—	—	273	72,9
81	2	—: 143	—	—	188	76,1
82	3	2: 4	1,5: 4,5	$0{,}70 > P > 0{,}50$	9	66,7
83	4	51: 134	46,3: 138,7	$0{,}50 > P > 0{,}30$	258	71,7
84	5	—: 37	—	—	48	77,1
85	6	71: 193	66: 198	$0{,}50 > P > 0{,}30$	343	77,0
86	7	11: 57	17: 51	$0{,}50 > P > 0{,}30$	94	72,3
87	8	57: 136	48,3: 144,7	$0{,}10 > P > 0{,}05$	264	73,1
89	10	70: 212	70,5: 211,5	$0{,}95 > P > 0{,}90$	405	69,6

Virido-lutescens-X-1. — A new type of fully vital chlorophyll aberration not easy to classify in previous systems. Recessives quite normal-green up to two weeks (1940)—four weeks (1941) before ripening date. The colour then rapidly turns yellow (CDC = 203), the chlorophyll components thus being destroyed. In terms of GUSTAFSSON's grouping (1940) the mutation should be classified as a *viridis* type, subgroup *lutescens*. Since the recessive, however, is characterized by normal-green colour for the majority of its growing period, the present writer has added the prefix »*virido-*» to the subgroup-name *lutescens*. It must be left as an open question whether such *virido-lutescens* types occur commonly enough to justify the establishing of a separate *virido-lutescens* subgroup. L. SMITH (1939) has in *Triticum monococcum* found two aberrant types »maple» and »golden» which could probably be classified as *virido-lutescens* types, although they keep more of the chlorophyll than does the *A. strigosa* mutation described. The height of the recessives of the *virido-lutescens-X-1* is only 80 % of that in normal-green homo- and heterozygotes. The recessive ripens about 5 days earlier than normal *A. strigosa*. Inheritance regularly monomeric.

Chlorina-X-1. — This type has been difficult to classify strictly and might even have been called »*lutescens*» in GUSTAFSSON's sense. The seedlings start yellow-green (CDC = 278) and gradually show deeper green colour. The development ceases at the 2-leaf stage, when a withering starts, giving an end-result much like that of the *lutescens* types. In the writer's opinion this new type should, however, not be classified as a *lutescens* but as a *chlorina*. Mode of segregation not determined.

Only 8 green plants and 3 *chlorina-X-1* recessives were found in the same X_2 progeny. The 8 green plants gave no segregation in X_3. The chloroplasts are smaller than in normal-green leaves (Figs. 4 and 5). Largest chloroplast diameter in the first leaf of *chlorina-X-1* (CDC = 301) at deepest green colour measured $3{,}1 \pm 0{,}052\ \mu$. In normal-green leaves of same age the corresponding value was $4{,}2 \pm 0{,}073\ \mu$ (Fig. 4, upper picture).

Tigrina-X-1. — A well-defined *tigrina* type. Viable, pollen good, seed-setting not markedly low, although the plants are not so tall and do not tiller so richly as do normal-green plants. Crosses have been carried out between this *tigrina-X-1* recessive and the spontaneously arisen *tigrina-1* reported in Ch. IV. Two fully normal-green F_1 plants were procured: hence the factors are not identical. The F_1 plants unfortunately never gave ripe harvest on account of insect attacks and drought. This statement on factorial difference could have been made, without the cross experiments, only with the help of the morphological observations and growth studies. The X-ray mutation is slightly less vital than the spontaneous *tigrina-1*. Segregations in field-grown X_3 progenies indicate a regular monomeric segregation without any complications.

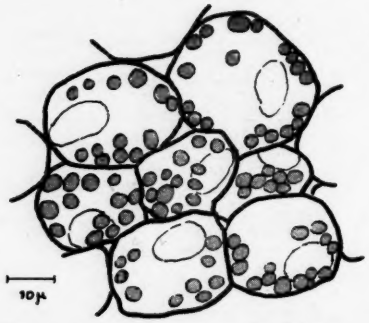

Fig. 5. Chloroplasts from mesophyll cells of the first leaf of *chlorina-X-1*. Corresponding normal-green leaf section, see Fig. 4.

Thus, in this X_2 comprising 152 plant-progenies 13 distinct chlorophyll mutations were obtained. The majority of the mutations (i. e. 8) were inherited monofactorially. If we refer the 13 mutations to the 152 plant-progenies, we get a total mutation frequency of $8{,}55 \pm 2{,}27$ %. The sampling error is calculated as $\pm\sqrt{\frac{13 \times 139}{152}}$. STADLER (1929, 1930) has calculated with mutation frequencies per single head-(panicle-)progeny, having used only the three first tiller heads of each X_1 plant (cf. STADLER, 1929, p. 878). The primordia of the three heads tested are separate in the embryo at the time of treatment: a mutation occurring at this time affects only a single culm. He has, however, also pointed out that »in much-tillered plants a secondary tiller often develops from an earlier tiller, and a single mutation may affect both» (1930, p. 3). In the present writer's opinion it would therefore be safer and more appropriate to refer the mutation frequency calculation to *the number of X_2 plant-progenies* (not head-progenies), and the same point of view is held by GUSTAFSSON in his barley experiments. For the sake of better comparison with STADLER's results the writer has, however, calculated the mutation frequency on the basis of the total number of panicle-progenies sown in X_2. Hence we get a total mutation frequency (243 X_2 panicle-progenies counted; it is then

purposely assumed here and in the following that no single mutation has affected more than one tiller) of $5{,}35 \pm 1{,}44$ % or, referred to single r-units (dosage 5.000 r): $(10{,}7 \pm 2{,}9) \times 10^{-6}$. As, moreover, only seldom were more than 3 panicles (or heads) developed in all investigated X_1 oat and wheat plants, we may rather safely consider the above assumption as practically justified.

In 1941 another X_2 was grown, partly from irradiated *A. strigosa* (the 5.000-r dose repeated; water-content 12,1 %; number of kernels treated 600), and partly from the also diploid *A. brevis* (water-content 10,8 %; 5.000 r; 600 kernels). The irradiation intensity was raised to 2.240 r/minute, i. e. to the 30-fold in comparison with that used for the series reported above. In this new series separate panicle-progenies were grown, and the sterility of all plant-progenies and panicle-progenies was determined from the X_1 thrashings (see p. 371). Since only few X_3 progenies were grown from these X_2 generations, the segregations of the new mutations have not been closely investigated.

The chlorophyll mutations thus obtained in *A. strigosa* were the following:

Albina-X-4. — 3 recessives found together with 29 green. A very rapidly withering and dying type. The X_1 panicle fertility 99,1. Fertility of the whole X_1 plant = 66,8 %.

Albina-X-5. — 2 recessives found together with 6 green plants. Distinctly more resistant to withering than the previous *albina* type. More vital. The X_1 panicle fertility 27,6 %; *this panicle originates from the same X_1 plant as above.* From the morphological and physiological differences between the *albina-X-4* and *-X-5* the writer must assume them to be genetically different mutations.

Albina-X-6. — 6 recessives found together with 8 green plants. Leaf tip with a characteristic rosa-lilac tinge. Dies at first-leaf stage. X_1 panicle fertility 46,1 %; fertility of the whole X_1 plant = 36,9 %.

Albina-X-7. — 4 recessives found together with 17 green plants. Wither off rapidly. X_1 panicle fertility 64,5 %; X_1 plant fertility 62,9 %.

Albina-X-8. — 1 recessive and 16 green plants found. X_1 panicle fertility 55,3 %; *this panicle originates from the same X_1 plant as gave the albina-X-7 above.* The former dies off rapidly, the latter persisted 6 days before withering. In the writer's opinion the *albina-X-7* and *-X-8* must be genetically different mutations.

Albina-X-9. — 1 recessive and 3 green plants found. X_1 panicle fertility 9,2 %. Developed a second leaf before withering off. X_1 plant fertility = 9,2 % (only one panicle).

Chlorina-X-2. — 1 recessive and 22 green plants found. Deepest yellow-green colour CDC = 253. Withers off at 2-leaf stage. Panicle fertility in X_1 = 87,6 %; this panicle originates *from the same X_1 plant as gave the albina-X-4 and -X-5 above.*

Chlorina-X-3. — 3 recessives and 6 green plants found. Viable up to 4-leaf stage. X_1 panicle fertility = 43,8 % (only one panicle on the X_1 plant). Deepest yellow-green colour CDC = 276.

Chlorina-X-4. — 5 recessives and 15 green plants found. Dies rapidly at 2-leaf stage. Deepest yellow-green colour CDC = 252. X_1 panicle fertility 59,9 %; X_1 plant fertility 52,3 %.

Chlorina-X-5. — 1 recessive, 1 green plant. X_1 panicle fertility = 4,6 % (only one panicle on the X_1 plant). Viable up to 2-leaf stage.

Chlorina-X-6. — 1 recessive and 3 green plants found. Viable up to 2-leaf stage

only. Deepest yellow-green colour CDC = 277. X_1 panicle fertility 9,2 %; X_1 plant fertility 36,9 %.

Chlorina-X-7. — 2 recessives and 8 green plants found. Dies at first-leaf stage. Maximal deepness of yellow-green colour CDC = 277. X_1 panicle fertility = 36,9 %. X_1 plant fertility = 18,4 %.

Chlorina-X-8. — 2 recessives and 8 green plants found. Dies at 3-leaf stage. Maximal deepness of yellow-green colour CDC = 253. X_1 panicle fertility 36,9 %; X_1 plant fertility = 25,8 %.

Chlorina-X-9. — 5 recessives and 10 green plants found. Dies at 5-leaf stage; might perhaps better survive a less dry spring than that of 1941. Maximal deepness of yellow-green colour CDC = 283. X_1 panicle fertility 41,5; X_1 plant fertility 47,7 %.

Chlorina-X-10. — 5 recessives and 8 green plants found. Dies at 2- or 3-leaf stage. Maximal deepness of yellow-green only CDC = 251. X_1 panicle fertility 52,3 % (only panicle on the X_1 plant).

Viridoalbina-X-3. — Very similar to the f. *albovirescens* (cf. Ch. IV). 2 recessives and 8 green plants found. Reaches 4—5-leaf stage, then withers. Base white, tip pure green (CDC = 301). Seedling leaves greening basewards. X_1 panicle fertility 36,3 % (only panicle on the X_1 plant).

Lutescens-X-6. — 1 recessive and 5 green plants found. Seedling dies on 1-leaf stage. X_1 panicle fertility 25,3 %; X_1 plant fertility 52,3 %.

Lutescens-X-7. — 1 recessive and 21 green plants found. 1st leaf normal-green; 2nd leaf displays yellow colour at leaf base. The seedlings then grow yellowish and die at 2-leaf stage. X_1 panicle fertility 82,9 %; X_1 plant fertility 65,4 %.

Xantha-X-1. — 3 recessives and 2 green plants found. Pure yellow (CDC = 227) during the whole seedling development. Dies at 2-leaf stage. X_1 panicle fertility 13,8 %; X_1 plant fertility 17,6 %.

Hence no fewer than 19 most probably genetically different chlorophyll mutations were obtained in this X_2. In all these experiments the untreated control did not yield any spontaneous mutations at all. In these field growings of X_2, sown panicle-progeny for panicle-progeny, the chance of 3 : 1 segregations appearing was not always favourable, since progenies comprising, for instance, only 8—10 seedlings were rather numerous. For the same reasons a dimeric segregation would hardly ever be found, not to speak of an, indeed very improbable, trimeric segregation. Since, however, the same difficulties are associated with all experiments carried out in the same manner by other authors, we are justified in comparing the data obtained with, for instance, those for barley and diploid wheat without making any special allowances for »mutations lost» on account of too restricted X_2 progenies.

The X_2 generation recorded gave from 600 irradiated X_1 kernels 281 seedlings (46,8 %) and at harvest 119 partly fertile plants, i. e. 19,8 %. The untreated control germinated to 87,8 %. The 119 X_1 plants produced 266 panicle-progenies, which yielded the 19 mutations recorded. We thus have a total mutation frequency (counted per panicle-progeny) of $7{,}14 \pm 1{,}58$ %, or, referred to single r-unit: $(14{,}3 \pm 3{,}16) \times 10^{-6}$. The agreement with the experiment series reported on pp. 364 ff. is thus very good.

The fertility percentages in the mutation list above were calculated as follows. A control series was counted (untreated material, 100 plants with 333 panicles; average panicle number 3,33). The following determinations were made: average number of kernels per plant (= 144,54), and average number of kernels per panicle (= 43,40). This last number was rounded off to 43 in calculating the sampling error. We then get N_p (number of kernels per panicle) in the untreated control = 43,40 ± 0,93 kernels.

In the *A. strigosa* treated with 5.000 r (2.240 r/min) we find for the X_1:

Average number of panicles per plant = 2,37
» » » kernels » » = 39,83
» » » » panicle = 16,81.

Rounding off the last figure to 17 in calculating the sampling error, we get N_{p_1} (number of kernels per panicle of X_1 plant) = 16,81 ± 0,87 kernels.

Now the average panicle fertility (kernels per panicle) is calculated as $N_{p_1} : N_p$. We find:

$$\text{Average panicle fertility of } X_1 = \frac{16{,}81}{43{,}40} = 38{,}7\ \%.$$

$$\text{Average plant fertility of } X_1 = \frac{39{,}83}{144{,}54} = 27{,}6\ \%.$$

If, for example, an X_1 plant has produced two panicles, the former with 26 kernels, the latter with 30, the panicle fertility is $26 : N_p$ and $30 : N_p$, i. e. 59,9 % and 69,1 %.

In the X_2 from irradiated *A. brevis* the following chlorophyll mutations were obtained:

Albina-X-1. — An X_1 plant comprised 9 panicles, one of which gave 6 kernels (panicle fertility 13,2 %). All 6 kernels gave *albina-X-1* seedlings that died at late first-leaf stage. X_1 plant fertility = 66,0 %. Possibly some selective fertilization had taken place here. The 6 recessives were very uniform in morphology and development. *Chloroplast studies revealed a few very faintly but distinctly green proplastids in some mesophyll cells*; compare Fig. 3, which illustrates rather well the plastid development in this *A. brevis albina-X-1* also.

Albina-X-2. — 2 recessives and 12 green plants found. X_1 panicle fertility 46,2 %; X_1 plant fertility 53,9 %. The recessives wither off at 2-leaf stage.

Albina-X-3. — 3 recessives and 10 green plants found. Tip red-stained. One plant reached 3-leaf stage before withering. X_1 panicle fertility 33,0 % (only one panicle on the X_1 plant).

Albina-X-4. — 3 recessives and 23 green plants found. X_1 panicle fertility = = 100,0 %. X_1 plant fertility = 58,8 %. Dies at late first-leaf stage.

Albina-X-5. — 6 recessives and 17 green plants found. X_1 panicle fertility = = 100,0 %; X_1 plant fertility 98,8 %. Dies very soon, at first-leaf stage.

Albina-X-6. — 3 recessives and 23 green plants found. X_1 panicle and plant fertility 100,0 %. Withers at 2-leaf stage.

Albina-X-7? — 7 recessives and 20 green plants found. Panicle and plant fertility in X_1 = 100,0 %. May be identical with the *albina-X-6* above so far as morphological observations permit any conclusion. Withers at 2-leaf stage. Originates from the same X_1 plant (another panicle) as *albina-X-6*.

Albina-X-8. — 2 recessives and 16 green plants found. Rapidly withering: dies at first-leaf stage in about 2—3 days. X_1 panicle fertility 94,6 %. X_1 plant fertility = = 41,8 %.

Albina-X-9. — 1 recessive and 22 green plants found. X_1 panicle and plant fertility = 100,0 %. Withered at first-leaf stage.

Chlorina-X-1. — 2 recessives and 7 green plants found. X_1 panicle fertility 22,0 %; X_1 plant fertility 12,1 %. Deepest yellow-green colour attained: CDC = 253. Dies at 3-leaf stage.

Chlorina-X-2. — 5 recessives and 12 green plants found. Second seedling leaf pure yellow (CDC = 207). Develops to 6-leaf stage but does not set seed. Carotinoids dominate, but obviously enough chlorophyll is present to permit a reduced assimilation. The 5th leaf shows CDC = 257 (yellowish-green). It is interesting to find that the size of the chloroplasts in sections from the 5th leaf of the recessive does not differ from that of normal-green *A. brevis* at the same stage (CDC = 327); Fig. 6. X_1 panicle fertility = 52,2 %; X_1 plant fertility = 37,4 %.

Tigrina-X-1. — 3 recessives and 1 green plant found. Not so well-defined morphologically as the *tigrina-X-1* of *A. strigosa* (p. 368) or the spontaneous *tigrina-1* of the same species (Ch. IV). Red-lilac transverse spots on the leaves, partly »pinched» leaves. X_1 panicle fertility 100,0 %; X_1 plant fertility = 66,0 %. Dies at 4-leaf stage.

With reference to the determination of sterility in the X_1 generation, unfortunately no control series was grown in 1940. In 1941 such a series was grown

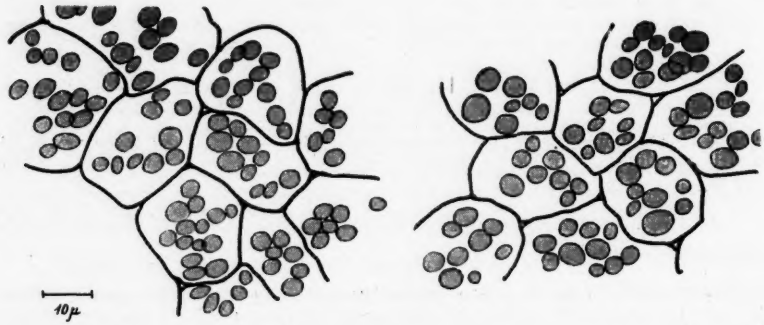

Fig. 6. Chloroplasts from mesophyll cells of the 5th leaf of *chlorina-X-2* (right) and of normal 5th leaf of *A. brevis* (left).

and analysed, and the figures obtained from this sterility analysis had to be used instead. The average panicle fertility of the *A. brevis* X_1 of 1940 was thus calculated at *73,0 %*, and the average plant fertility would then be nearly the same, viz. *73,8 %*. In spite of the control not being grown in corresponding years there is an obvious indication that the 5.000-r treatment has not caused such a deep depression of the fertility in *A. brevis* X_1 1940 as in *A. strigosa* X_1 1940, this being in good agreement with the lower water-content of the *A. brevis* seeds at the time of treatment (cf. Table 4).

Thus, in the *A. brevis* X_2 generation we have found 12 (11?) chlorophyll mutations. The number 12 presupposes that the *albina-X-7* above is counted as genetically different from the *albina-X-6*, which is, however, a mere supposition. Per 244 separately grown panicle-progenies in X_2 we would then find a frequency of $4{,}92 \pm 1{,}39$ %, or, referred to single r-unit: $(9{,}8 \pm 2{,}8) \times 10^{-6}$, i. e. a fairly good agreement with the frequency obtained above in the other irradiated oat diploid for the same dosage (5.000 r).

3. EXPERIMENTS WITH TETRAPLOID OATS.

Returning to Table 4, p. 362, the tetraploid oat species *A. barbata* and *A. abyssinica* were to some extent investigated as to the X_1 seedling germination. The former species is not well adapted for field growings, since the ripening is too late under Svalöf conditions: practically no full-ripe harvest can be had from even untreated control plots, and far less from still later ripening X_1 generations. In 1942 an X_2 comprising 88 *A. barbata* panicle-progenies from No. 1941—951 r 36 (5.000 r) and 43 *A. barbata* plant-progenies from 951 r 34 (same dosage) was grown in the field. No chlorophyll mutations at all were obtained. Reliable determinations of sterility were impossible to undertake on account of the too unsatisfactory ripening of the 1941 harvest. A small X_2 from *A. barbata* No. 1941—951 r 37 (dosage 10.000 r) comprised only 20 plant-progenies, obviously originating from rather sterile X_1 plants; no chlorophyll mutations were obtained. As to the earlier ripening *A. abyssinica* (1943—963—r 1 and 2) an X_2 was obtained only from the 5.000-r treatment, comprising 71 plant-progenies. No chlorophyll mutations, however, segregated in this X_2, although quite clearly the 71 X_1 plants were strongly influenced by the irradiation.

As regards resistance to X-ray treatment, the tetraploid oats investigated obviously occupy an intermediate position between the diploids and hexaploids (cf. in Table 4 especially the column for 10.000 r).

4. INDUCED CHLOROPHYLL MUTATIONS: HEXAPLOID OATS.

The 9 investigated varieties (and species) of hexaploid oats in Table 4 might be supposed to show an equally great resistance to X-ray induction of chlorophyll mutations as did the above reported tetraploids. This was however true only for the varieties Engelbrekt II, Abeds Nova and Sapeli. As for the species *A. fatua* and *A. sterilis*, the X_1 plants were unfortunately too retarded in development and displayed too bad a seed-setting to permit any X_2 generation to be grown, and they must hence be omitted from the discussion. In Seger, Guldregn I and Klock II *from one to three distinct chlorophyll mutations were found.* The variety Stormogul I, finally, gave in the X_2 generation 1942 the surprisingly high number of *12 chlorophyll mutations*, many of them no doubt regularly monomerically inherited — and this from a 10.000-r treatment, i. e. not an extreme X-ray dosage. Such an astonishing feature in a hexaploid can hardly be interpreted otherwise than on the assumption that Stormogul I for some reason has accumulated a considerable number of non-reduplicated chlorophyll factors as compared with the other varieties tested (cf. Ch. VII: Discussion). Sterility determinations of X_1 in all varieties tested have been undertaken (exception: Seger 1943—963 r 6—9). On account, however, of the extremely dry vegetation season in 1941, which caused a most heterogeneous development even in neighbouring plots, these determinations are unfortunately rather valueless, and hence more detailed references to the X_1 sterility will be omitted. A brief record follows of the mutations found.

(1) 500 kernels of the variety Seger were irradiated with 10.000 r

in 1941. The X_1 generation comprised 184 seedlings, giving rise to 62 panicle-carrying plants, these in their turn giving only 53 panicles with kernels. Hence the X_1 sterility was very pronounced. One of the X_2 panicle-progenies segregated into 1 *tigrina-X-1* : 5 green. In X_3 none of the 5 green plants repeated the segregation. The recessive was very characteristic: the first leaf was transversally striped in pure white. Between the white areas the colour was a pure normal green (CDC = 301). The transverse leaf contraction in the white bands was quite pronounced. The recessive withered after 5 days at first-leaf stage.

Another X_2 panicle-progeny segregated into 5 *viridoalbina-X-1* : 13 green. In X_3 the supposed monomeric segregation was regularly repeated (found 38 : 99; expected $34{,}3$: $102{,}7$). The recessive withers at first-leaf stage or may attain early 2-leaf stage. Base white, tip green (CDC = 306).

(2) The variety Klock II was given 10.000 r in 1941 (500 kernels irradiated). The X_2 generation comprised 245 plant progenies, grown as 512 separate X_2 panicle-progenies. One of these panicle-progenies segregated into 5 *virescens-X-1* : 35 green. Subsequent X_3 tests proved the segregation to be monomerical with a marked deficit of recessives. Found numbers in X_3: 279 *virescens-X-1* : 1132, the expectation for a monomeric ratio being $370{,}3$: $1040{,}7$. The recessive dies off at 4-leaf stage. The colour is then CDC = 301—302; at 1- and 2-leaf stage the colour is light green (CDC = 311—312).

(3) The variety Guldregn I was treated with 10.000 r in 1941. The X_2 generation comprised 145 plant-progenies, grown as 252 separate X_2 panicle-progenies. As above, the X_1 resulted from 500 treated kernels.

The X_2 panicle-progeny 967—25 segregated into 4 *chlorina-X-1* : 17 green. The *chlorina* recessive resembled the spontaneous *chlorina*, found by ÅKERMAN (cf. ÅKERMAN and FRÖIER, 1941); it is viable and sets a few seeds. In direct comparison with the spontaneous mutation, grown side by side the next year, the *chlorina-X-1*, however, displays a more retarded development and a deeper colour at earlier stages. Cross experiments to prove the genetical difference of the induced mutation from the spontaneous have failed. There are, however, in the author's opinion, very small chances indeed of a genetical identity existing between these two *chlorina* types. In X_3 a very regular monomeric segregation was obtained.

Another X_2 panicle-progeny, 967—26, originating from the same X_1 plant, segregated into 3 *chlorina-X-2* : 4 green. The recessive closely

resembles the *chlorina-X-1* in all but one respect, viz. the colour at earlier stages is not so deeply yellowish-green. In the X_3 of the next year the seedlings of spontaneous *chlorina* matched CDC 252—257, those of *chlorina-X-2* did about the same, and those of *chlorina-X-1* reported above corresponded to CDC 282. Hence the *chlorina-X-2* shows more resemblance to the spontaneous *chlorina* than does the *chlorina-X-1*; a genetical identity between the two former was not readily assumable on account of the more retarded development of the *chlorina-X-2*. Here, only a cross experiment could furnish definite proof. On account of the low seed-setting such cross experiments, undertaken in 1943, unfortunately failed (*chlorina* × *chlorina-X-1*, *chlorina* × *chlorina-X-2* and, finally, *chlorina-X-1* × *chlorina-X-2*). The segregation of the *chlorina-X-2*, tested in X_3 in 1944, was quite regular and monomeric.

A third X_2 panicle-progeny, 967—42, showed a segregation of a typical *tigrina* form, called *tigrina-X-2*, because a *tigrina-X-1* had already been found in hexaploid *A. sativa* oats (Seger; cf. p. 374). The *tigrina-X-2* plants found were 3 comparatively vigorous seedlings, viable up to 5—6-leaf stage and displaying the same characteristic transversal striping as the *tigrina-X-1* of *A. strigosa*. An anatomical study revealed similar features of chlorophyll destruction. Together with the 3 recessives, 17 green plants were found. In the X_3 of the next year none of these 17 plants showed any *tigrina* segregation, which leaves the mode of inheritance quite unexplained.

(4) 500 kernels of the variety Stormogul I were irradiated with 10.000 r in 1941. The X_1 generation comprised 384 seedlings, giving rise to 183 tillered plants, the latter in their turn yielding 552 separate panicle-progenies in X_2. In comparison with Seger oats above the X_1 sterility was thus not very marked — a good illustration of the effects of the extremely dry season of 1941. The variety Stormogul is decidedly more drought-resistant than Seger.

The following chlorophyll mutations were obtained:

Albina-X-1. — 1 recessive and 48 green were found. Segregation unknown; among 36 X_3 progenies grown partly in the field, partly in sand dishes, only two segregated (1 : 79 and 3 : 41 respectively). Dies rather slowly, not before early 2-leaf stage.

Viridoalbina-X-2. — 2 recessives and 3 green found. In the X_3 the 3 green gave two non-segregating progenies and one segregating 17 *viridoalbina-X-2* : 70 green, thus indicating a monomeric ratio with deficit of recessives. The recessive has a white base; tip green (CDC = 306—307). Dies off at 3-leaf stage.

Virescens-X-2. — 3 recessives and 8 green found. The green seedlings gave only 2 mature plants, which did not repeat any segregation in X_3. The recessives died off at 3-leaf stage.

Virescens-X-3. — 17 recessives and 21 green found. Segregation repeated in

X_3 seems to indicate a monomeric ratio with marked deficit of recessives (found 41 : 366; expected 101,8 : 305,2). Withers off at 4-leaf stage.

Virescens-X-4. — 1 recessive and 2 green found. Segregation not repeated in X_3. The recessive withered off at 3-leaf stage.

Chlorina-X-3. — 2 recessives and 3 green found. Segregation not repeated in X_3. The recessives die at 3-leaf stage. Maximal deepness of yellow-green colour CDC = 277.

Lutescens-X-1. — 4 recessives and 20 green found. Only two X_3 progenies from the 20 green plants have given manifest segregations (3 : 35 and 2 : 141 respectively), which does not allow of any conclusions as to the mode of inheritance. Withering typically at 1—2-leaf stage.

Lutescens-X-2. — 1 recessive and 1 green plant found; the latter did not repeat the segregation in X_3. The recessive withered off rapidly at first-leaf stage, turning bright whitish-yellow.

Lutescens-X-3. — 3 recessives and 12 green plants found. Segregation probably monomeric with a slight deficit of recessives; found in X_3 altogether 20 : 101; expected 30,3 to 90,7. Withers off at early 2-leaf stage.

Lutescens-X-4. — 6 recessives and 22 green found. In 8 segregating X_3 progenies altogether 21 recessives were found on 690 green. Segregation was thus greatly disturbed by deficit of recessives. Recessives wither off at 2-leaf stage.

Lutescens-X-5. — 2 recessives and 21 green found. An entirely regular monomeric segregation was found in altogether 12 segregating X_3 progenies (expected values 184 recessives : 552 normal-green; found 180 : 556). The recessive dies at 2-leaf stage.

Lutescens-X-6. — 3 recessives and 43 green found. Mode of segregation not ascertained on account of too great sterility of the green X_2 plants. The recessives wither off at 2-leaf stage.

The mutations in Stormogul I recorded above — totalling 12 — give a calculated frequency of 12/552 mutations per panicle-progeny, i. e. $2{,}17 \pm 0{,}62$ %, or, referred to single r-unit: $(2{,}17 \pm 0{,}62) \times 10^{-6}$.

As regards the X_1 generations from higher dosages (cf. Table 4), the resulting X_2 generations were greatly limited on account of the extreme X_1 sterility. No chlorophyll mutations have been observed in these X_2 generations.

Observations on the occurrence of *induced fatuoids* have been made in all X_2 generations recorded above. Not a single fatuoid has been observed as yet, but further studies on the new X_2 generations repeated annually from now on will be necessary before definite conclusions can be drawn.

Another rather remarkable feature in the observations on these hexaploid oat X_2 generations is that, so far, in the black-hulled oats investigated (Klock II, Stormogul I, Engelbrekt II) at least no obvious increase in *the mutation frequency of black kernel colour to grey or white* seems to have appeared as a result of the X-ray treatment. It should be noted, however, that no special observations have been carried out in order to compare strictly induced and spontaneous mutation frequencies of kernel colour mutation, the induced chlorophyll aberrations being of primary interest to the author.

Before any case of mutated chlorophyll factors with polymeric inheritance was discovered, oat grain colour mutations from black to grey or white were well-known. NILSSON-EHLE (1907 a, 1911 a), for instance, showed that there could be no question of spontaneous crosses as an explanation of the grey-grained plants found in the black oats Klock and Stormogul. Subsequent researches in the Seed Control Department of the Swedish Seed Association (briefly reported by ÅKERMAN and FRÖIER, 1941, p. 399) showed that the spontaneous mutation rate in the common monofactorial black oats Engelbrekt II and Stormogul II from central Sweden was as high as 1 : 2.000.

As regards *other morphological and physiological mutations* in X_2, the hexaploid oats have distinctly responded to the 10.000-r treatment with a great many genomatic re-arrangements, leading to segregations of new types, varying in length of straw, tillering capacity, earliness, leaf width, hairiness of nodes, etc. These new forms will be described in later publications.

VI. INDUCTION OF X-RAY CHLOROPHYLL MUTATIONS IN DI-, TETRA- AND HEXAPLOID WHEAT.

1. MATERIAL.

This chapter chiefly reports induced chlorophyll mutations obtained from diploid *Triticum monococcum* L. and tetraploid *T. dicoccum* SCHÜBL. In four different hexaploid wheat varieties tested no chlorophyll mutations have so far been obtained from dosages up to 20.000 r, although 6 large X_2 generations from successfully grown X_1 plants have been obtained; both spring and winter varieties have been tested. Likewise, no chlorophyll mutations were obtained from the tetraploid *T. durum* DESF. (10.000 r dosage). In Table 5 the various field-grown X_1 wheat generations from 1939—1944 are given together with percentages of seedlings germinated, numbers of sown kernels, water-content and provenience of irradiated seeds.

Respecting the technique of irradiation, see Ch. V, 1. The intensity of X-raying was from 2.200—2.500 r/min, except for the 1939 *T. monococcum* series, where 72 r/min was used, and for the 1944 Skandia III series, where 870 r/min was employed on account of the high water-content of the treated seeds (19,4 %). The induced chlorophyll mutations are — as in oats — recorded in numerical order for each species.

The diploid *T. monococcum* used belongs to the v. *Hornemanni* KÖRN., the tallest and most commonly cultivated variety of the species. The *T. dicoccum* belongs to the var. *farrum* KÖRN. (White Russian Emmer). The *T. durum* v. *hordeiforme* KÖRN. is one of the most widely distributed varieties of the species, grown in Russia and Asia Minor; the majority of the *durum* wheats grown in the United States belong to this variety. The diploid and tetraploid wheats thus irradiated were all spring forms. Among the *vulgare* wheats irradiated, Diamant II originates from the cross Diamant I × Extra-Kolben II, the former from a cross between a land wheat from Halland and Svalöf's Kolben, the latter from a cross German Emma-wheat × Svalöf's Kolben. Kolben wheat originates from German Heines Kolben and has in all probability exactly the same origin as the well-known American Fife wheat.

TABLE 5. *Percentage of seedlings counted in X_1 from X-rayed di-, tetra- and hexaploid wheats. X_1 grown in the field.*

(Numbers in brackets = kernels sown.)

Field number	Species, variety	Water-content %	Seed provenience	Untreated control, % seedlings germinated	Percentage of seedlings germinated				
					5.000 r	10.000 r	15.000 r	20.000 r	30.000 r
	Diploids:								
	T.monococcum								
1939—959 —r 1	v. *Hornemanni* KÖRN.	12,1	1938	—	—	6,9 (1.000)	—	—	—
1939— 1459	»	12,1	1938	88,3 (150)	—	—	—	—	—
1941—951 r 67—70	»	10,9	1940	—	46,0 (500)	0,0 (500)	0,0 (500)	0,0 (500)	—
r 71	»	10,9	1940	71,1 (90)	—	—	—	—	—
	Tetraploids:								
1941—951 —r 63— 65	*T. dicoccum* SCHÜBL. v. *farrum* KÖRN.	11,3	1939	—	—	52,6 (500)	—	1,2 (500)	0,0 (1.000)
r 66	»	11,3	1939	72,2 (90)	—	—	—	—	—
1941—951 —r 59— 61	*T. durum* v. *hordeiforme* KÖRN.	12,1	1939	—	—	42,2 (500)	—	0,0 (500)	0,0 (1.000)
r 62	»	12,1	1939	70,0 (90)	—	—	—	—	—
	Hexaploids:								
	T.vulgare HOST								
	Spring forms:								
1941—951 —r 51— 53	Diamant II	11,0	1939	—	—	31,0 (500)	—	0,0 (500)	0,0 (1.000)
r 54	»	11,0	1939	75,6 (90)	—	—	—	—	—
1941—951 —r 55— 57	Kolben	12,0	1940	—	—	4,6 (500)	—	0,0 (500)	0,0 (1.000)
r 58	»	12,0	1940	74,4 (90)	—	—	—	—	—
1943—963 —r 10— 12	Kolben	11,1	1942	—	—	69,4 (500)	20,4 (500)	3,6 (1.000)	—
r 13	»	11,1	1942	80,0 (100)	—	—	—	—	—
1943—963 —r 14— 16	Progress	11,2	1942	—	—	63,2 (500)	18,0 (500)	4,6 (1.000)	—
r 17	»	11,2	1942	73,0 (100)	—	—	—	—	—

Field number	Species, variety	Water-content, %	Seed provenience	Untreated control, % seedlings germinated	Percentage of seedlings germinated				
					5.000 r	10.000 r	15.000 r	20.000 r	30.000 r
1943—963									
—r 18—20	Diamant II	10,9	1942	—	—	57,0 (500)	16,2 (500)	1,1 (1.000)	—
r 21	»	10,9	1942	88,0 (100)	—	—	—	—	—
	Winter forms:								
1942—966									
—r 2, 3, 12, 13	01144 [1]	12,8	1940	—	—	—	79,7 (1.000)	36,1 (1.000)	—
r 1	»	12,8	1940	80,0 (50)	—	—	—	—	—
1942—966									
—r 5, 6, 10, 11	Gluten [1]	13,1	1940	—	—	—	83,3 (1.000)	29,4 (1.000)	—
r 4	»	13,1	1940	86,0 (50)	—	—	—	—	—
1942—966 —r 8—9	Skandia III [1]	13,2	1940	—	—	—	78,0 (1.000)	34,3 (1.000)	—
r 7	»	13,2	1940	78,0 (50)	—	—	—	—	—
1944— 2063— 2066	Skandia III [2]	19,4	1943	—	58,4 [3] (1,000)	75,6 (1.000)	60,4 (1.000)	41,7 (1.000)	—
2062	»	19,4	1943	63,2 (1.000)	—	—	—	—	—

Progress, finally, originates from a cross Ä 23/8 × Extra Kolben II, the former line being obtained from the cross Svalöf's Kolben × German Red Schlanstedter. Among the winter wheats 01144 is derived from the cross Kron × Bore II, Skandia III from Kron × Fylgia, and Gluten from (Pansar I × Thule II) × Svea II.

As in oats (Ch. V), it is obvious that the tetra- and hexa-ploid wheats are more resistant to X-ray treatment than the diploids. The X_1 generations from 1941 were unfortunately exposed to severe drought; from Table 5 it can be seen that the hexaploid varieties Diamant II and Kolben grown as X_1 in 1943 show a considerably larger number of seedlings at dosages from 10.000 r onwards than do the same varieties in 1941. The X_1 generations grown in 1942 in soil pots in the greenhouse which were then planted in the field gave a still larger seedling number. The X_1, finally, from Skandia III in 1944 showed an equally large seedling number; the overwintering conditions were this year very favourable.

2. INDUCED CHLOROPHYLL MUTATIONS: DIPLOID WHEAT.

Induced mutating in diploid wheat was first recorded by STADLER (1929). In *Tr. monococcum* L. the types »white» (2 cases), »virescent»

[1] X_1 first grown in soil pots in the greenhouse, each pot containing one treated kernel. The pots with viable seedlings were then planted in the open field during normal autumn-sowing time. The percentage numbers refer to overwintered seedlings.
[2] X_1 sown in the field. The percentage numbers refer to overwintered seedlings.
[3] Dosage 7.500 r.

(1 case) and »yellowish-green» (3 cases) were found. Even at an X-ray dosage of 5.800 r-units (intensity = 290 r/minute) *T. monococcum* was so severely injured by the exposure that the mutation frequency could not be determined on account of too few X_1 plants being available. For a dosage of 2.900 r (= 10 min. exposure) STADLER found an average mutation frequency (of all mutations counted together) of $(10{,}4 \pm 3{,}4) \times 10^{-6}$, which was probably higher than that of Himalaya barley, $(4{,}9 \pm 0{,}9) \times 10^{-6}$. In STADLER's material we thus find a first indication that there does not exist any difference in mutation frequency of at least certain mutable genes (chiefly chlorophyll genes studied) in diploid wheat and barley.

Later on L. SMITH (1936) began genetical and cytological studies of *Tr. monococcum* and *Tr. aegilopoides* in order to lay a foundation of knowledge of these non-polyploid species as a basis for later studies of the effects of polyploidy in existing polyploid species of *Triticum* and in artificially induced amphidiploid hybrids. With the aid of X-rays SMITH produced (1939) among 400 mutations over 80 viable ones. It is stated by SMITH that »the lethals (white and yellow seedlings or other inviable types) and types poorly separable from normal have been discarded» (l. c., p. 3). Among SMITH's »viable mutations» we find enlisted the following chlorophyll mutations: argentia-a and -b, argentia c, creamex-1 and -2, creamex 3, finestripe-a, finestripe-b, green base, glossy creamex, golden, green veins, japonica, jadestripe, light green, light stripe, lightex, maple, streak, yellow, yellow green, yellowex-1 and -2, and yellow zoned.

It has been possible for the author to identify at least the chief groups of mutation types in these chlorophyll aberrations, briefly described by SMITH, with the type-groups of the maize geneticists, of DE HAAN (1933) and of GUSTAFSSON (1940). SMITH's investigations partly offer very valuable conclusions as to the number of chlorophyll genes in diploid wheat. The complexity of the genetic back-ground for the chlorophyll organisation is obvious. The normal chlorophyll development can apparently be arrested by a great many different chemical processes both below and above the chromophoric threshold. Just as in barley and maize, mutations of chromophoric and hypo-chromophoric types are comparatively easy to produce in diploid wheat by means of X-rays.

The present writer, before learning of SMITH's investigations, had already started X-raying experiments with *T. monococcum*. In 1939 1.000 kernels with a water-content of 12,1 % were given 10.000 r-units

(intensity = 72 r/min). The untreated control germinated to 88,3 %. Only 69 seedlings (= 6,9 %) were obtained in the field in 1939 from these 1.000 treated kernels, and at harvest only 37 plants with mature spikes (= 3,7 %) could be thrashed individually. Determination of plant and spike sterility was not undertaken in this investigation. In 1940 the X_2 from the 37 X_1 plants was sown. The following distinct chlorophyll mutations were obtained in the X_2:

Albina-X-1. — 6 recessives found together with 44 normal-green seedlings. On dying develops slight yellow tinge. Withers rather slowly off. Probably monomerically inherited.

Albina-X-2. — 2 recessives and 3 green plants found. Dies rapidly at first-leaf stage without any yellowish transitional stages. In X_3 a progeny segregating 1 recessive : 34 green was obtained. Ratio of segregation unknown.

Albina-X-3. — 5 recessives and 2 green plants found; the latter did not repeat the segregation in X_3. Persists to 2-leaf stage and may even develop a third, entirely white leaf. Chloroplast studies show only quite colourless or the faintest yellow plastids of very small size.

Virescens-X-1. — 3 recessives and 11 green plants found. Segregation not repeated. Seedlings start whitish-green (CDC = 311). The 3rd and 4th leaves are deeper green: CDC = 304—305 — the development then ceases, and the seedlings die off.

Lutescens-X-1. — 2 recessives and 2 green plants found. Segregation was not repeated in X_3. The recessives died off at 1- or 2-leaf stage.

Lutescens-X-2. — 3 recessives and 3 green plants found. In X_3 a single segregating progeny was obtained with 9 recessives and 25 green plants. Probably monomeric segregation. Seedlings wither and die rapidly at young first-leaf stage.

Lutescens-X-3. — 2 recessives found together with 5 green plants. Segregation in X_3 in one progeny 5 *lutescens-X-3* : 12 green. First seedling leaf yellowish with yellowish-green tip (CDC = 277), second leaf rosa-yellow, withering in whitish-yellow colours. Third leaf does not develop.

Thus, in this X_2 seven chlorophyll mutations were obtained from 37 plant progenies. The mutation frequency of diploid oats (see Ch. V) was based on the number of panicle-progenies in X_1, this being done to get better comparison with earlier authors. In the *T. monococcum* X_2 generation referred to above separate head-progenies were not grown, the heads of each X_1 plant having been thrashed together. However, at the harvest of the X_1 a record was made how many head-bearing tillers were likely to set seed in the next generation, and in this way 48 tillers were counted, i. e. 1,3 wholly or partly fertile heads per plant. Thus, the 7 mutations can be referred to 48 head-progenies, which gives a chlorophyll mutation frequency of $14{,}6 \pm 5{,}1$ % or, if we refer this frequency to r-units, $(14{,}6 \pm 5{,}1) \times 10^{-6}$. This high frequency indicates that diploid wheat is by no means inferior to barley in respect to induced chlorophyll disturbances; see Discussion in Ch. VII.

3. INDUCED CHLOROPHYLL MUTATIONS: TETRAPLOID WHEAT.

In the tetraploid wheat *Triticum dicoccum* SCHÜBL. v. *farrum* KÖRN. 500 kernels were irradiated with 10.000 r; water-content 11,3 %; intensity 2.240 r/minute. The resulting X_1 generation was grown in 1941 and comprised 263 seedlings, giving 158 mature plants with — after harvest — 369 more or less fertile heads. The X_2 was sown in 1943, the total material of 1942 being too extensive to permit the X_2 generation in question to be included. Two distinct chlorophyll mutations were obtained:

Albina-X-1. — 1 recessive and 9 green plants found. The segregation was repeated in X_3 the next year. Four segregating progenies comprised in all 63 recessives : 246 normal-green, i. e. a slight deficit of recessives. Expected numbers would be 77,3 : 231,7 with $\chi^2 = 3{,}528$ and $0{,}10 > P > 0{,}05$. The recessive persists up to 2-leaf stage.

Viridoalbina-X-1. — A very well-defined mutation. 3 recessives and 22 green plants found. The segregation was repeated in X_3 the next year. 10 segregating and 7 non-segregating progenies were obtained. The former totalled 163 recessives : 577 normal-green plants. Expected numbers for a 3 : 1-ratio would be 185 : 555 with $\chi^2 = 3{,}488$ and $0{,}10 > P > 0{,}05$. The deficit of recessives can be accounted for by inferior sprouting ability, just as in the *albina-X-1* above. The *viridoalbina-X-1* recessives have the first leaf base pure white, tip green (CDC = 301); the 2nd leaf is characteristically tubuliform (leaf-margins adhere). Dies at advanced 2-leaf stage.

Both mutations were obtained from rather sterile X_1 heads and X_1 plants respectively.

The high effect of the 10.000-r dosage on the species *T. durum* v. *hordeiforme* KÖRN. is shown in Table 5. The higher dosages in this 1939 series did not yield any viable X_1 seedlings at all. From the 10.000 r dosage (500 kernels treated) 211 X_1 plants resulted, grown in 1941, these giving rise to 77 mature plants with, after harvest, 101 more or less fertile heads. The corresponding 101 X_2 progenies, grown in 1942, did not exhibit a single chlorophyll aberration in spite of repeated careful scrutinies. The fertility of the X_1 plants, defined as kernel number per plant, was 15,0; that of the untreated control was 45,2. Hence the X_1 plant fertility can — roughly — be put down as *33 %*. In the species *T. dicoccum* v. *farrum* above the X_1 plant fertility was somewhat higher, *41 %*. In both tetraploid species the X-ray treatment consequently brought about corresponding depressions of the X_1 plant fertility — note the somewhat higher water-content of the irradiated *T. durum* seeds (Table 5) as probably partly responsible for the lower X_1 plant fertility in this species.

4. EXPERIMENTS WITH HEXAPLOID WHEAT.

As is seen from Table 5, the irradiation experiments with spring and winter forms of *T. vulgare* have been carried out on a rather

comprehensive scale: altogether 20.000 kernels were irradiated up to 1944. Certain practical restrictions, however, led to only the 6 seemingly most promising X_1 generations being grown as X_2. For the same reasons only few sterility analyses were undertaken. In the X_2 generations grown a most careful field (and greenhouse) scrutiny has been made of the seedlings — up to four times during the early plant development — in order to detect even slight chlorophyll aberrancies; in sharp contrast to the experiments with hexaploid oats (Ch. V) *no induced chlorophyll mutations at all were found in the hexaploid wheat varieties studied.*

The experimental data gathered from the various X_2 generations studied are recorded below, the same order being followed as in Table 5: Hexaploids.

(1) *Diamant II* (spring wheat), *10.000 r.* — 500 irradiated kernels yielded 109 mature plants, resulting in an X_2 in 1943 with 352 separate head-progenies. The fertility of the X_1 plants was *76 %* of the corresponding untreated control plants. In this X_2 no obvious segregation of speltoids, compactoids or distinctly morphologically aberrant forms was observed. It was, however, noticed that a certain increased variability in ear density and length of straw was conspicuous in comparison with the untreated material. The X_2 was discarded at harvest, and it was decided to repeat the irradiation in a subsequent season.

(2) *Kolben* (spring wheat), *10.000 r.* — 500 irradiated kernels yielded only 16 mature plants, resulting in an X_2 during 1942 with 25 separate head-progenies. The average X_1 plant fertility was *21 %*. Among the 25 progenies 3 segregated in speltoid-like types and 2 in ear-density and length of awns respectively.

(3 a) *Kolben* (spring wheat), *10.000 r.* — 500 irradiated kernels yielded an X_1 in 1943, comprising 347 seedlings, giving 207 mature plants. These were thrashed individually, but separate head-progenies were not grown in X_2. The average X_1 plant fertility was *43 %*. In the X_2, grown in 1944, 89 among the 207 X_1 plant-progenies displayed a segregation in distinct speltoids and speltoid-like types, i. e. no less than 43 *%*. A thorough classification of the speltoids and speltoid-like types has just been commenced. However, as the field classification in 1944 was carefully done, only comparatively few mistakes can be suspected. The variety studied further responded to the irradiation with a marked segregation into several types, aberrant in ear-density, leaf-width and length of straw, the study of which is now in progress. The untreated control was here represented by single plant-progenies

from the X_1 control; among 64 such progenies none displayed a single speltoid or otherwise morphologically aberrant type.

(3 b) *Kolben* [the same seed provenience as in (3 a)], *15.000 r.* — 500 irradiated kernels yielded 102 X_1 seedlings in 1943, giving only 33 mature plants. As in the above case, separate head-progenies were not grown. The average X_1 plant fertility was *24 %*. In the X_2, 1944, 12 among the 33 progenies (36,4 *%*) segregated into speltoids and speltoid-like types. Besides these, segregations were observed in length of straw, heading-time and tillering, all in comparison with the variability in the 64 untreated control progenies recorded above under (3 a).

(4) *Diamant II* (spring wheat), *10.000 r.* — 500 irradiated kernels yielded an X_1 in 1943, comprising 285 seedlings that gave 100 mature plants. As before, separate head-progenies were not grown. The average X_1 plant fertility was *77 %*. In X_2, grown in 1944, 46 among the 100 X_1 plant-progenies segregated in speltoids and speltoid-like types, this giving a percentage of not less than 46 [cf. (3 a) above]. Several segregations of straw-stiffness, earliness, extremely long awns, non-waxy hulls, denser ears, etc. were also observed in this X_2. The untreated control consisted of 36 single plant-progenies from the X_1 control. These 36 progenies were uniform and homogeneous, not showing a single morphologically aberrant type.

(5) *Gluten* (winter wheat), *20.000 r.* — 1.000 irradiated kernels were sown in as many small soil-pots and put in the greenhouse for subsequent planting in the field. The yield was 294 seedlings. Of these, only the first portion, 232 seedlings, was afterwards used, the second portion (62 seedlings) being too retarded in development to give any fertile plants at harvest. The first portion yielded 61 mature plants, which were thrashed. Separate head-progenies were procured in this X_1: hence the X_2 of 1943 consisted of 280 progenies. The average X_1 plant fertility was *52 %*; the untreated control (28 single plant-progenies) exhibited no segregation into aberrant types. The 280 progenies in X_2, however, showed 37 segregating families: 1 in length of straw, 11 in compactoids, 10 in speltoids or speltoid-like types, 11 in other ear-density aberrants and 4, finally, in awnness. Thus, altogether 13,2 *%* of the X_2 families disclosed a very obvious irradiation effect in comparison with the untreated control.

(6 a) *Skandia III* (winter wheat), *15.000 r.* — 1.000 irradiated kernels yielded an X_1 in 1944, comprising 604 overwintered seedlings. Practically all these seedlings gave mature plants. Unfortunately, the soil at harvest was so hard and crusty that the extraction of single

plants from the plot failed in part — a certain although low number of plants were thus involuntarily divided into pieces. Hence the X_2 of 1945 consisted of 617 families, the vast majority of which, however, were complete and undivided X_1 plant-progenies. Among the 617 X_2 families 52 segregated into fertile or ± sterile speltoids or speltoid-like types (8,4 %), 2 in compactoids, 2 in awnness and some 10 families in straw-length, earliness and other morphological aberrations, now selected for further investigations. The average X_1 plant fertility was 79 %; the untreated control comprised no fewer than 311 families (single plant-progenies from the X_1 control). In this rough fertility calculation it may be assumed that the control plants in the X_1 suffered from involuntary division in the same degree as did the X_1 15.000-r plants. The 311 control families in the X_2 exhibited some phenotypic variation, although no speltoids, compactoids and other aberrations similar to those found in the irradiated X_2 were observed.

(6 b) *Skandia III* [the same seed provenience as in (6 a)], *20.000 r.* — 1.000 irradiated seeds yielded an X_1 in 1944, comprising 417 overwintered seedlings. For the same reasons as were reported under (6 a) above, the X_2 of 1945 numbered 427 families, the majority of which, however, must be assumed to consist of complete (undivided) X_1 plant-progenies. Among the 427 families 61 segregated into fertile or ± sterile speltoids or speltoid-like types (14,3 %), 4 in compactoids, 2 in awnness and some 15 in other morphological aberrations, at present being subjected to investigation. X_2 control = the same 311 families as recorded above.

The significance of these totally negative results from induction of chlorophyll mutations in hexaploid wheat is discussed in Ch. VII.

VII. DISCUSSION.

In the foregoing chapters the scarceness of spontaneous chlorophyll mutating in oats and wheat has been stressed on the basis of the reports hitherto published (Introduction and Chapter I). Chapters II and III present the results of investigations on the *chlorina* and *lutescens* factor distribution in several hexaploid oat varieties and species. These results have been discussed in detail under the respective chapter headings. Three new spontaneous chlorophyll oat mutations are described in Chapter IV. In Chapters V—VI, finally, a considerable number of induced chlorophyll mutations are reported, obtained from irradiated kernels of diploid and hexaploid oats, diploid and tetraploid wheat.

The genetic back-ground for the development of a normal chlorophyll organisation in plants must at present be considered as very complicated. Most probably we have to deal with a vast number of interacting genes, influencing a long chain of reactions. As soon as any one of these genes is changed by gene-mutation or replaced or eliminated by structural alterations the equilibrium of the reaction mechanism is influenced. If the gene action is quantitatively insignificant, the equilibrium will not be disturbed to such a degree as to produce any visible chlorophyll aberration under normal conditions. The existence of such genes must — indirectly — be inferred from, e. g., HALLQVIST's and COLLINS's reports on barley *albina* mutations, which develop normally green above a certain temperature (Ch. I, p. 311). In hexaploid oats the present writer has found a f. *pallida* (as yet unpublished) in which the chlorophyll deficiency is extremely slight and in some years not even perceptible; in all probability it must, however, be denoted as a spontaneous, monofactorially inherited mutation.

In the majority of known chlorophyll aberrations the final reaction equilibrium is more or less altered. It may be reached below or above the chromophoric threshold. In barley and maize, for instance, the considerable number of non-identical *albina* allels found (cf. GUSTAFSSON, 1940, p. 31) clearly proves that the chlorophyll organisation can be broken down at many different points below the chromophoric threshold, where carotinoids and chlorophylls cannot even be formed. Special interest attaches to the writer's *albina-X-1* mutation from *Avena strigosa* (Ch. V), a transitional type, where the anatomical study revealed the existence of small, scarce but undoubtedly green plastids of varying size. Here the chlorophyll development must have been arrested just on the threshold of further greening. It is possible that such a recessive mutation might be induced to advance its greening some few steps further under altered light and temperature conditions (cf. RANDOLPH's discussion on similar types in maize, 1922).

If the reaction equilibrium is reached above the chromophoric threshold, several types of chlorophyll aberrations appear, with carotinoids or chlorophylls dominating in the plant development. In GUSTAFSSON's practical system of nomenclature for barley mutations (1940, p. 4) the groups II—VII can with slight adjustments serve as an adequate key of classification for these wheat and oat chlorophyll deficiencies as well. GUSTAFSSON's system will therefore be henceforth used in this discussion.

It is seen from Ch. I that the previous literature reports on *spont-*

aneous chlorophyll deficiencies in oats and wheat deal with aberrations belonging to most hitherto known general mutation groups. In the first line *albina* types have been observed (probably in 50 % of the Mendelian inherited cases), but true representatives of the *xantha*, *alboviridis*, *viridis* and *tigrina* types have in all probability also been met with. Among the cases of non-Mendelian inherited spontaneous aberrations the *striata* group is well represented. In oats and wheat the *maculata* group has its first known representative in the f. *luteomaculata* of hexaploid oats, described in Ch. IV (not included in Table 1, since it has not been reported until now).

In Ch. I NEATBY's note (p. 310) respecting JOHNSON's observations on diploid *Triticum monococcum* attracts primary attention. For the first time we are here confronted with an obviously higher spontaneous chlorophyll mutation frequency in this cereal than was before known. In diploid oats corresponding frequencies are not known, owing to the limited interest the diploid varieties possess in modern agriculture and plant-breeding. Thus, in the field observations at Svalöf only one spontaneous chlorophyll mutation has hitherto been reported in diploid oats (f. *tigrina*; Ch. IV).

As to the frequency of *induced* chlorophyll mutation in diploid wheat and oats, STADLER (1929, 1930) was the first to show that this frequency is decidedly higher than in polyploid wheat and oats. The present results gathered from irradiation experiments by several authors (L. SMITH, GUSTAFSSON, FRÖIER) afford, in fact, no reasons for supposing any real difference to exist in chlorophyll gene mutability between barley, diploid oats and diploid wheat. In Table 6 the total chlorophyll mutation frequencies from STADLER's, GUSTAFSSON's and FRÖIER's experiments with these diploids are collected.

In Table 6 the mutation frequencies have been calculated *per* X_1 *head-(panicle-) progeny* and r-unit. GUSTAFSSON's results in barley originally refer, however, to the *number of* X_1 *plants, not to the* X_1 *head-progenies*. At 10 % water-content he found a frequency of (on an average for 5.000 and 10.000 r) $10{,}1 \times 10^{-6}$, and at 15 % a frequency of about $15{,}4 \times 10^{-6}$ mutations per X_1 plant and r-unit. To obtain a better comparison with STADLER's frequency calculations, GUSTAFSSON's frequencies have been halved in Table 6 on the rather probable assumption of about 2 heads per X_1 plant. It is obvious that in this comparison of frequencies the order of magnitude occupies the first line of interest; a more exact comparison will require many repeated irradiations of the same material.

TABLE 6. *Induced chlorophyll mutation frequency in barley, diploid oats and wheat.*

Author	Species and strain tested	Intensity, r/minute	Dosage, r-units	Water-content, %	Chlorophyll mutation frequency per head- (panicle-) progeny and r-unit
STADLER (1929)	Himalaya barley	290	2.900		
			5.800		
			8.700		$(4{,}9 \pm 0{,}9) \times 10^{-6}$
			11.600		
STADLER (1929)	*Tr. monococcum*	290	2.900		$(10{,}4 \pm 3{,}4) \times 10^{-6}$
GUSTAFSSON (1940)	Gull barley	72	5.000	10 %	$(5{,}0 \pm ?) \times 10^{-6}$
			10.000		
GUSTAFSSON (1940)	Gull barley	72	5.000	15 %	$(7{,}7 \pm ?) \times 10^{-6}$
			10.000		
FRÖIER (Ch. VI)	*Tr. monococcum*	72	10.000	12,1 %	$(14{,}6 \pm 5{,}1) \times 10^{-6}$
FRÖIER (Ch. V)	*Avena strigosa*	72	5.000	12,6 %	$(10{,}7 \pm 2{,}9) \times 10^{-6}$
FRÖIER (Ch. V)	*Avena strigosa*	2 240	5.000	12,1 %	$(14{,}3 \pm 3{,}2) \times 10^{-6}$
FRÖIER (Ch. V)	*Avena brevis*	2 240	5.000	12,3 %	$(9{,}8 \pm 2{,}8) \times 10^{-6}$

When comparing the frequencies of *Triticum monococcum*, *Avena strigosa* and *A. brevis* with those of Himalaya and Gull barley we find that the diploid wheat and oats in question are decidedly not inferior to barley in respect to the total frequency of induced chlorophyll deficiencies. Besides, recent calculations by GUSTAFSSON (in the press), based on all his barley investigations up to now, clearly show an average total frequency of induced chlorophyll mutations which does not exceed the values for wheat and oat diploids in Table 6. In STADLER's results from Himalaya barley the mutation frequency tabulated is a medium for the dosages 2.900, 5.800, 8.700 and 11.600 r; the water-content of the irradiated kernels was not reported.

As regards the different types of induced mutations and their relative proportions in these diploids, the following may be stated from the writer's experiments. *Albina* types occur abundantly in X-rayed oat and wheat diploids. In the *Triticum monococcum* series they constituted 43 %, in *Avena strigosa* (both series reckoned together) 28 %, and in *A. brevis* 75 (73?) % of all mutations. This is in fair conformity with the proportions in barley: according to GUSTAFSSON (1940, p. 23) induced and spontaneous *albina* mutations constitute 40—50 % of all chlorophyll mutations.

Chlorina and *lutescens* mutations also frequently occur. In *A. strigosa* the *chlorina* proportion was considerable (31 %); in *A. brevis* this type represented 17 % (18?) of all mutations. *T. monococcum* has so far not yielded any typical *chlorina*, although the chief *viridis* group, to

which this type belongs, is represented by 57 % *lutescens* and *virescens* mutations in this diploid (3 *lutescens* mutations, 1 *virescens* produced). In *A. strigosa* 25 % *lutescens* mutations were found (the »*virido-lutescens-X-1*» on p. 367 then also being included); *A. brevis* did not display any *lutescens* type. On account of the considerable proportion of *chlorina* and *lutescens* mutations found it is not astonishing that one of the first recorded spontaneous chlorophyll mutations in hexaploid oats was just a *chlorina* (Ch. I and II) and, further, that a typical *lutescens* aberration was also among the first to be observed and closely investigated in this hexaploid (Ch. I and III).

The chief *alboviridis* group is represented with 9 % (3 cases) of all mutations in *A. strigosa*, these 3 cases belonging to the subgroup *viridoalbina*. In the other diploids no mutations of this group have been met with.

The chief *xantha* group was represented only in *A. strigosa* with one single but typical mutation. The very interesting *tigrina* group has had two representatives: one in *A. strigosa* and one in *A. brevis*. Mutations of this type are comparatively rare in barley. A notable fact is that 2 typical *tigrina* mutations were also obtained from irradiated hexaploid oats (see below). Finally, no representatives of the *striata* and *maculata* groups have been observed.

As seen from Table 6, the dosage, intensity of irradiation and seed metabolism have perceptibly varied. With our present state of knowledge the comparisons made are, however, sufficiently conclusive, as at these dosages the dosage proportionality may be considered as practically linear. The different water-contents of the treated seeds and other unknown differences in seed metabolism can, of course, favour certain mutation types — the general lines of comparison, however, will not be greatly disturbed by these incongruences. The fact that certain typical barley chlorophyll mutations have not yet been found in these irradiated diploids cannot therefore invalidate the general conclusion that *wheat and oat diploids are on an average equally susceptible to induction of chlorophyll mutations as is barley*. This fact is now decisively established: for diploid wheat chiefly by L. SMITH's investigations (Ch. VI, p. 380) and for diploid oats by the present writer (Ch. V).

A further question is, of course, if this conclusion is also valid as regards the *spontaneous* mutability. Can the mechanism of induced mutation be assumed merely to represent an acceleration of the spontaneous mutation processes or not? Among others, STADLER has advanced important critical considerations upon this crucial point

(1932, p. 293): In all work with induced mutation it must be borne in mind that the results may be generalized »only on the assumption that mutations in general are a homogeneous class, of which the induced mutations are a representative sample». The great difficulty — seldom to be overcome in practice — is the experimental test of the validity of this assumption: it requires the determination of specific mutation rates, which may be extremely low. This has been done for 8 endosperm genes in maize (STADLER, 1932); the frequency of any specific chlorophyll gene mutation has as yet never been determined.

In *A. strigosa* (the second series in Table 6 with 12,1 % water-content) and *A. brevis* the X_1 plant and panicle fertility have been determined. In spite of the limited material and the relative uncertainty of these fertility determinations (chiefly due to attacks of the frit fly in X_1) some of the gathered data may be briefly mentioned. The distribution of *albina* mutations over the different ranges of X_1 panicle sterility was different in the two species. In *A. strigosa* the *albina* mutations are distributed over the whole fertility range; the fertility percentages were in ascending order 9,2 — 27,6 — 46,1 — 55,3 — 64,5 and 99,1. The average value is 50,3 %. In *A. brevis* with its high total *albina* frequency we find a tendency to accumulation in the higher fertility classes: 13,2 — 33,0 — 46,2 — 58,8 — 94,6 — 100,0 — 100,0 — 100,0 and 100,0 (the *albina-X-7*, p. 371, then also included). The average value is 71,8 %. The *albina* distribution in both diploid oat species does not contradict GUSTAFSSON's experiences of the distribution of induced *albinas* in barley. The present material is, however, too limited to give effective support to his general conclusions on the intragenic nature of *albina* mutations (cf. GUSTAFSSON, 1940, pp. 23 ff.). The distribution of *viridis* mutations in the *A. strigosa* series (9 *chlorina* + 2 *lutescens* mutations) gave the following fertility percentages: 4,6 — 9,2 — 25,3 — 36,9 — 36,9 — 41,5 — 43,8 — 52,3 — 59,9 — 82,9 and 87,6. The average value is 43,7 %. When reckoning both *Avena* species together we find a manifest difference indicated by the distribution of *albina* and *viridis* mutations over different fertility classes: in the highest fertility class (90—100 % fertility of X_1 panicles) 6 of 15 *albinas* are found. None of the 13 *viridis* mutations obtained are found in this fertility class.

As regards the vitality of the 51 chlorophyll mutations reported above from diploid oats and wheat only 2 are vital (*virido-lutescens-X-1* and *tigrina-X-1* in *Avena strigosa*) and 1 sublethal (*chlorina-X-2* in *A. brevis*). The others are lethal types, developing but 1—4 leaves

before withering. No dominant mutation was found. Regular monomeric segregation could be proved for 8 of the 51 cases, while for the major part of the rest such a segregation could be considered as rather probable.

Turning to the irradiation experiments with tetraploid wheat and oats, it is a deplorable fact that these experiments were too limited to permit any important conclusions. The tetraploid oats investigated yielded no mutations at all; among the two tetraploid wheats irradiated the *T. dicoccum* v. *farrum*, however, yielded two distinct chlorophyll mutations, a typical *albina* and an equally well-defined *viridoalbina*. Both mutations were lethal, monomerically inherited with a deficit of recessives, and were obtained from rather sterile X_1 heads.

As mentioned in the introduction, STADLER has stated that in tetraploid wheat and oats »a considerable proportion of the mutable genes appear to be dominant only in one of the chromosome groups». This statement was then supported by the findings of 1 mutation in *T. dicoccum* and 6 mutations in *T. durum* at dosages of 2.900—11.600 r. All segregations were apparently monogenic. The present writer's results from *T. dicoccum* (10.000 r) can only serve to support STADLER's statement on this point; the negative results from *T. durum* must no doubt be regarded as the work of mere chance.

A certain proportion of mutable, non-reduplicated genes in wheat tetraploids being thus established, the question arises whether the same holds true for tetraploid oats. No induced chlorophyll mutation has so far been reported for oats with 28 chromosomes. This question, however, must be definitely answered in the affirmative, partly for reasons of analogy with the findings in tetraploid wheat, partly on account of the astonishingly high mutation frequency found in certain hexaploid oat varieties (see below).

In irradiation experiments with *autotetraploids*, first with barley (MÜNTZING, 1942) and secondly with flax (LEVAN, 1944) not a single chlorophyll mutation was produced in spite of the dosage being raised up to 25.000 and 35.000 r-units respectively. Hence the strict genomatic reduplication has here effectively prevented any such mutations from appearing. The chance of obtaining dimerically segregating mutations in an *allotetraploid* as a result of a »double-hit» ($A_1A_1\ A_2A_2$ transformed to $A_1a_1\ A_2a_2$) is obviously extremely small — not to speak of the chances of getting trimerically segregating mutations in hexaploids. In an *autotetraploid* recessive individuals $a_1a_1\ a_1a_1$ have, it is true, better chances to appear as offspring from heterozygotes $A_1A_1\ A_1a_1$.

In MÜNTZING's and LEVAN's experiments, however, no such recessives were found in spite of the rather comprehensive material studied.

Proceeding to the results from the irradiation experiments with hexaploid oats and wheat, we find the new and interesting fact that *it has been possible to induce a considerable number of chlorophyll mutations from hexaploid oats* (Ch. V). *In hexaploid wheat, however, it has been impossible to induce any chlorophyll mutations at all, even at dosages ranging up to 20.000 r* (Ch. VI). From the hexaploid oat varieties Seger, Klock II, Guldregn I and Stormogul I altogether 18 chlorophyll mutations were procured, the dosage used being then only 10.000 r.

Beginning with hexaploid oats, literature reports on experiments with X-ray-induced mutations are lacking. In fact, since STADLER's paper of 1929 nothing seems to have been published on this subject. The chlorophyll mutations found in the present investigations belong to most of the typical groups recorded above. Only in few cases could their inheritance be established with any safety: the greater part seems, however, to constitute monomerically segregating types. But other mutations obviously segregate in proportions indicating considerable cytological disturbances and aberrations from the normal genomatic structure. The most interesting feature is no doubt met with in the variety Stormogul I, where no fewer than 12 chlorophyll mutations were found from a 10.000 r dosage, the chlorophyll mutation frequency here being $(2{,}17 \pm 0{,}62) \times 10^{-6}$, i. e. of the same order of magnitude as in the diploids (cf. Table 6). The average X_1 panicle fertility was *51 %* in this Stormogul I : 10.000-r series. The corresponding fertility values for Seger, Klock II and Guldregn I were *28 %*, *55 %* and *60 %*. Sterility analyses were not performed for the remaining hexaploid oats.

The majority of the 18 chlorophyll mutations from hexaploid oats belong to the *viridis* group (13 mutations). 2 *alboviridis* types and 2 typical *tigrina* mutations were obtained; only one true *albina* was found. As for the *viridis* mutations found, a tendency can be traced in their distribution over the X_1 plant fertility range: most *viridis* types seem to originate from highly sterile X_1 plants. This coincides with the experiences of GUSTAFSSON, who mentions (1940, p. 27) that in barley extreme sterility favours the origin of *viridis* mutations. Two of the chlorophyll mutations obtained were vital (*chlorina* types) and one sublethal (*tigrina-X-2*), the rest lethal. No dominant mutation was found.

In the paper by FRÖIER, GELIN and GUSTAFSSON, 1941, it was shown that in *Avena sativa* the amount of induced chromosomal disturbances

in X_1 was at 10.000 r as high as about 43 % (percentage of disturbed cells). The water-content at irradiation has been practically the same in the present experiments. It can hence be safely concluded that the 10.000 r-dosage has caused sufficient minor and major chromosome disturbances to explain the considerable number of mutations produced in the X_2 of hexaploid oats. The various morphological and physiological mutations obtained will be discussed in later publications, solely the chlorophyll mutations being of interest to us at present.

As already touched upon above, *the number of induced chlorophyll mutations obtained from a polyploid must be supposed to roughly reflect the number of non-reduplicated chlorophyll factors in the polyploid in question.* Although little is known of the intra- and inter-genic alterations induced by the irradiation of these polyploids, it is obvious that intragenic change, replacement or elimination of non-reduplicated chlorophyll genes must in the first line be responsible for the appearance of the monomerically segregating chlorophyll mutations found. As for mutations the segregation of which has not been established in the present investigation, the possibility of any hidden dimeric segregations seems practically negligible. No indications of such ratios have so far been met with.

The general reasoning above on the connection of the induced mutation number with the number of non-reduplicated chlorophyll factors in the polyploid oats has one chief difficulty to deal with: *specifically high-stable non-reduplicated chlorophyll genes may defy even the heaviest X-ray dosages applicable. The proportion of such genes will therefore remain unknown.* In spite of this consideration, we are justified in considering the total induced chlorophyll mutation frequency as representative of the number of non-reduplicated genes *possessing average stability* — in other words, of the »basic mutation rate» of the polyploid (cf. STADLER, 1929, p. 881).

This reasoning explains the relatively great space devoted to the *chlorina* and *lutescens* factorial distribution in Chapters II and III. The results of these investigations ought to have contributed to the mutation problem in polyploid oats and wheat and perhaps furnished the general discussion with some new aspects. Since in hexaploid oats a considerable number of varieties have been shown to exhibit only one *chlorina* factor or one *lutescens* factor as a result of previous successive steps of mutation from the three-factorial stage, we are justified in assuming *that for other chlorophyll factors in hexaploid oats monofactorial varieties also occur to a certain, as yet unknown extension.*

The first interesting evidence of the validity of this argument is offered by the results from Stormogul I. Here 12 most probably genetically different chlorophyll mutations — many of them monomerically segregating — were procured at only a medium sterility degree of the X_1 plants and medium X-ray dosage.

It must be stressed that the chance variation between various X_2 generations of the same variety and subjected to the same X-ray treatment is of course considerable. If it had been possible, for instance, to repeat the treatments of the Stormogul I pure line for some ten years in succession — with identical metabolic conditions of the seeds — the variation in number and type of chlorophyll mutation would probably have been very beautifully illustrated. At present it is impossible to judge the limits of this variation. It must, however, be assumed that in respect of the proportion of non-reduplicated genes Stormogul I may probably differ from the other hexaploid oats irradiated, which have partly yielded but few mutations (Seger, Guldregn, Klock II) and partly no mutations at all.

The above conclusions from hexaploid oats are in sharp contrast with those to be drawn from the irradiation experiments with wheat hexaploids. Previous authors have dealt with such experiments, partly on a fairly large scale. The first work reported was by DELAUNAY (1930), who used irradiated ears (not seeds) as material. In subsequent papers (1931, 1932, 1934) DELAUNAY reports the production of a great many mutations from these treatments, the majority of which consist of speltoids or speltoid-like types, compactoids, dwarfs, »cylindroids» and »agropyroids» together with several undefined mutations. DELAUNAY's report does not permit any conclusion as to X-ray intensity or dosage suitable for a comparison with the results of the present work. Nowhere, however, in DELAUNAY's reports are any chlorophyll mutations mentioned.

Shortly after DELAUNAY, in 1930, A. A. SAPEHIN published a preliminary note on irradiation experiments with hexaploid wheat plants just before flowering (same method as DELAUNAY); a more concise report is found in his paper of 1935. Both spring and winter varieties of *Triticum vulgare* were used; the dosages employed were low (about 2.500 r). The mutations produced were on the whole in conformity with DELAUNAY's type-groups. No chlorophyll mutations were recorded, however. AFANASSJEWA (1936) subjected *T. vulgare* var. Caesium O. III to a cytological study with similar designs to those of FRÖIER,

GELIN and GUSTAFSSON (1941) but did not advance the investigation to studies of the X_2 segregation.

FONDARD and CABASSON in 1939 briefly report experiments with X-raying of pollen of the hexaploid winter wheat variety »Tuzelle» (old pure line from Provence). No chlorophyll mutations were produced. The dosages used were not reported. A great many morphological mutations were, however, found in the following generations. RANJAN (1940) irradiated Pusa wheat but found no chlorophyll aberration among some few morphological mutations produced.

SCHKWARNIKOW, working with mutations induced by seed ageing, was the first to report induced chlorophyll mutations in hexaploid wheat (1936 a, 1937, see also 1939). In his paper of 1936 a vital, light-green mutation is briefly mentioned, probably a *viridis* type. The paper of 1937 briefly reports some chlorophyll mutations (number and type not mentioned) obtained from 7—10 years old seeds. The great majority of these mutations consisted of speltoids, sterile types and dwarfs. Respecting speltoids, compactoids, etc., see the complete and reliable survey of the literature by UCHIKAWA (1941).

In the present investigations no chlorophyll mutations at all have so far been obtained, in spite of the rather heavy dosages used (up to 20.000 r) and the number of varieties tested. The great number of induced speltoids, compactoids, other morphological and physiological mutations in these hexaploid wheats, together with the knowledge of the great many chromosome disturbances normally induced in the dosage range 10.000—20.000 r (FRÖIER, GELIN and GUSTAFSSON, l. c.), leads unquestionably to the primary conclusion that *in hexaploid wheat the proportion of non-reduplicated chlorophyll genes must be very small.* Since in wheat *diploids* a great many chlorophyll genes can easily be induced to mutate, it can hardly be objected that extremely stable, non-reduplicated genes may in general be responsible for the scarceness of induced chlorophyll mutations in polyploid wheat. The lack of factorial distribution studies on any of the few chlorophyll aberrations reported in hexaploid wheat (cf. Ch. I) prevents further conclusions from being drawn as yet. That interesting question, whether or not reduplicated genes may mutate with different frequencies owing to their different genic environment in the various genomes, cannot be discussed here but should be borne in mind in future works on the subject.

Reverting, finally, to the apparently very great stability of the chlorophyll apparatus in the polyploid wheat and oats, the gene re-

duplication must doubtless be regarded as the main cause of this stability. For hexaploid wheat the assumption of STADLER (1929, p. 881) that »apparently the proportion of mutable genes not reduplicated is very small in the species with the triple chromosome number» seems at present wholly justified. For hexaploid oats it can no longer be upheld. The experiments conducted up to now speak distinctly in favour of a less thorough chlorophyll gene reduplication in this polyploid. The reasons for the apparent difference between these polyploids can hardly be subjected to any fertile discussion before more evidence has been gathered from future experiments. The degree of allo- or autopolyploidy in the investigated genera is still too insufficiently known to permit any conclusions.

As to conclusions of any importance for practical plant-breeding the following may be said. In hexaploid wheat the appearance in X_2 of a vast number of speltoids, compactoids, straw-length mutations, etc. will indicate a successful X-ray treatment; chlorophyll mutations can only exceptionally be supposed to segregate. In hexaploid oats such mutations will probably not infrequently be met with, the segregation of morphological mutations in X_2 being here on an average not so obvious to the eye as in wheat. An actual question of great practical importance is, to what extent genes inhibiting extremely early development are reduplicated in oats and hence not easily accessible for induced mutation experiments. As a matter of fact, certain practical experiences gathered up to now indicate greater possibilities of inducing early mutations in oats than seem to be offered in wheat.

SUMMARY.

(1) The present investigations comprise a genetic study of the distribution of the *chlorina* and *lutescens* factors in different hexaploid oat varieties and species, reports on three new spontaneous chlorophyll mutations in hexaploid and diploid oats, and an account of a large number of such mutations produced by irradiation of di-, tetra- and hexaploid oat and wheat varieties. In addition, certain anatomical and physiological properties of the mutations produced have in some cases been preliminary investigated.

(2) In hexaploid oats repeated mutations of the homologous, polymeric *Chlor* factors must have occurred in the phylogenetic course of development. This successive mutating has not advanced so far in the black-hulled central-Swedish oats as in the Probsteier varieties investig-

ated. The spontaneous mutation frequency *Chlor* → *chlor* must be very small, as (a) many market strains are monofactorial and up to now merely one case of mutation has been observed, and (b) many trifactorial varieties seem still to exist.

(3) Similar conclusions are drawn from the *lutescens* factor distribution. Oats of the same ecological group show the same set of homologous, polymeric *L* factors. The varieties Mesdag and Line 29/100 from Dalslandshavre carry a previously unknown *L* factor set representing a second transitional stage between tri- and monofactorial constitution. Gene-physiological observations on the *L* factor action make it highly probable that these unquestionably homologous chlorophyll factors act upon the mechanism of chlorophyll formation in identical manner, seemingly independent of the genetic environment. Hence the *lutescens* case in hexaploid oats presents the most cogent argument hitherto adduced for factorial reduplication identity caused by polymery.

(4) A new spontaneous chlorophyll mutation, f. *albovirescens*, belonging to the chief *alboviridis* group (GUSTAFSS.), was found in hexaploid oats (Chapter IV). Another spontaneous mutation of the *tigrina* type was found in the diploid *Avena strigosa*. The inheritance is regularly monomeric. The new type is semivital and holds an intermediate position in vitality between the lethal induced barley *tigrina* types and the only slightly subvital »zebra» types found in maize (Chapter IV). This spontaneous *tigrina* mutation attracts special interest because no less than four *tigrina* mutations were produced from irradiated oats: one in *A. strigosa* (fully vital), one in *A. brevis* (lethal) and two in hexaploid oats (lethal — sublethal); Chapter V. A third spontaneous chlorophyll aberration, f. *luteomaculata* was, finally, found in hexaploid oats (Chapter IV). It originates most probably from a spontaneous plastid mutation and is exclusively maternally inherited. Its characteristic yellow leaf dots are caused by a decomposition of the chloroplasts in the mesophyll cells; the chloroplast-destroying processes are here, however, not accompanied by lack of mesophyll cell-turgor as in the *tigrina* types, where transversal deturgescence of the leaf as a rule appears.

(5) Irradiation experiments with dry seeds of oat and wheat diploids together with the results of previous authors in diploid wheat and barley, show that these diploids respond to low-medium X-ray dosages by a considerable number of chlorophyll mutations. These belong to the most known groups of barley mutations, tabulated accord-

ing to the practical system of GUSTAFSSON (1940). They are all recessive and mostly monofactorially inherited. Many morphological and physiological mutations also appear (ear-density, awnness, earliness, length of straw, etc.), and these will be dealt with in future communications.

(6) The average total chlorophyll mutation frequency of diploid oats and wheat is not inferior to that of barley. Altogether 51 chlorophyll mutations were obtained from diploid oats and wheat at dosages of 5.000—10.000 r. For frequency comparisons, see Table 6.

(7) Only a limited number of irradiation experiments were conducted with tetraploid oats and wheat. Their results do not, however, contradict previous assumptions on the plausible frequency of non-reduplicated genes in these polyploids (STADLER, 1929).

(8) Similar experiments on a fairly large scale with hexaploid wheat and oat varieties gave the following results. In *oats* altogether 18 recessive chlorophyll mutations were obtained at but medium dosages (10.000 r) and medium X_1 sterility (28—60 %), the black-oat Stormogul I yielding most of these mutations. In *wheat* no chlorophyll mutations at all appeared even at 20.000 r dosages and considerable X_1 sterility. A vast number of speltoids, compactoids and other morphological and physiological mutations were, however, found in these wheat series as evident signs of the great many chromosome alterations and re-arrangements induced. Further evidence on this point was gathered by FRÖIER, GELIN and GUSTAFSSON in a previous paper on the cytological response of polyploidy to X-ray dosage (1941).

(9) The comparisons so far carried out between hexaploid oats and wheat speak distinctly in favour of the conception that in oats a higher percentage of non-reduplicated chlorophyll factors exists. This conception is further supported by the findings made in the studies of the distribution of the *chlorina* and *lutescens* factors reported in Chapters II and III. The causes of this dissimilarity between the hexaploid oat and wheat varieties investigated must be left undecided for the present.

(10) The general assumption of gene reduplication as the main cause of the scarce occurrence of chlorophyll mutations in polyploid oats and wheat (STADLER, 1929) is supported by the present results, inasmuch as induced mutating of the chlorophyll genes can be supposed to represent a roughly uniform acceleration of the corresponding mutation processes. An important new feature is, however, the apparent difference exhibited in chlorophyll factor mutability between hexaploid

wheat and oats — a fact that is also of interest for the future work with induction of practically valuable mutations in these polyploids.

(11) The complexity of the genic background of the chlorophyll development is discussed. Among results from the present work the *albina-X-1* mutation from *Avena strigosa* should be mentioned as an interesting transitional type, the chloroplast development being arrested just on the threshold of further greening.

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INFLUENCE OF TEMPERATURE ON THE NUCLEOLUS AND ITS COACERVATE NATURE

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ON studying mitosis the function and properties of the nucleolus must be clarified. The regular emergence of the nucleolus at telophase and its disappearance during prophase show that it is morphologically directly connected with mitosis. Several authors, especially older ones, have considered this periodical cycle to indicate that the spindle is made up directly of nucleolar substance (TISCHLER, 1942).

The purpose of the present paper is to demonstrate the influence of temperature variation on the size of the nucleolus in the resting stage and on its disappearance during prophase.

MATERIAL AND METHODS.

The material consisted of root meristems of cuttings from one tree of *Salix fragilis* × *alba*. To prevent genetical factors from disturbing the effect of temperature, cuttings belonging to the same clone were studied (see below). As soon as the roots had grown out the cuttings were put into dark, thermostatically controlled chambers permitting of temperatures ranging from 10° to 30° C. (Within this range growth and cell divisions are quite normal.) Otherwise the methods of cultivation and preparation were analogous to those described by the author (1946) in a study on the influence of temperature on the spindle. As in that investigation, the roots were fixed in chrome-acetic-acid formalin and the sections were stained in iron-aceto-carmine.

RESULTS.

1. *The nucleolar cycle.* — The nucleolar cycle of *Salix* should be briefly discussed before reporting the results of the experiments. In the resting nucleus of a root meristem cell of *Salix* one nucleolus regularly appears. As a rule it is almost spherical and frequently it is

optically homogeneous. Sometimes the interior of the nucleolus is of a lighter shade. Most likely the method of preparation is responsible for the irregular occurrence of this phenomenon. In early prophase, perhaps even still earlier (cf. DOUTRELIGNE's measurements, cit. by TISCHLER, l. c., p. 198), the nucleolus begins to diminish and as a rule has completely disappeared at the end of prophase. Sometimes, however, it persists in the same or in slightly altered magnitude until metakinesis and even until metaphase. Persisting nucleoli seem to be especially

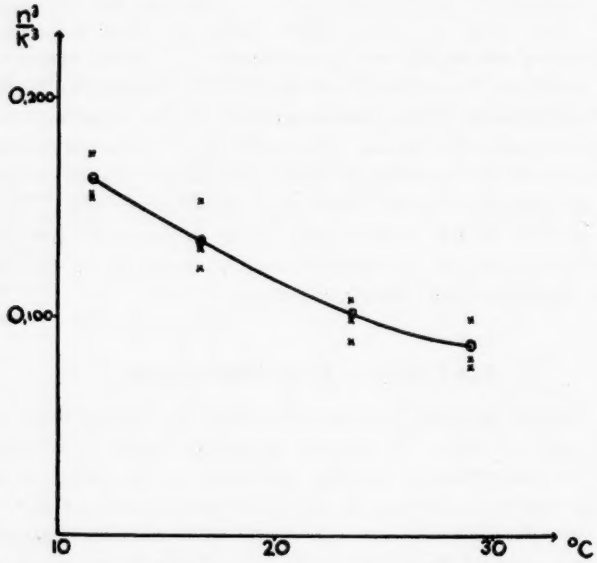

Fig. 1. Diagram showing the relative size of the nucleolus as a function of temperature. ⊙ = temperature mean value; × = root mean value.

common in certain *Salix* species, and within the variable *Salix phylicifolia* L. they have been observed only in some races to occur in great numbers. Although the plants were not cultivated under controlled light and temperature conditions in these experiments, a fact which makes statistical data rather useless, the author believes that in *Salix* the point in the nuclear cycle at which the nucleolus disappears is genetically controlled. In order to facilitate the interpretation of the influence of temperature on the investigated phenomenon, the experiments were performed on a genotypically homogeneous material.

2. *The size of the nucleolus.* — To represent the variation in

TABLE 1. *Influence of temperature on the size of the nucleus and the nucleolus. Experiment data and covariance analysis.*

Temp. °C	Root No.	Number of measurements (N)	Mean value of k ($\bar{k}$) units	Mean value of x ($\bar{x}$)	$\frac{\bar{n}}{\bar{k}}$	$b\frac{n}{k}$	r	$t = \sqrt{\frac{(N-2)\,r^2}{1-r^2}}$	$s = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N-1}} \cdot 100$	$\frac{s}{\bar{x}}$
11,5	442	26	22,10	0,174						
	451	13	24,85	0,156						
	462	15	23,61	0,154						
	Σ	54	23,18	0,163	0,54	0,54	0,8623	12,3***	3,94	0,242
16,5	411	14	24,17	0,153						
	452	17	19,79	0,131						
	453	17	20,72	0,122						
	Σ	48	21,40	0,134	0,51	0,58	0,8680	11,8***	3,46	0,258
23,5	402	12	20,31	0,089						
	433	25	20,03	0,108						
	443	11	22,22	0,099						
	Σ	48	20,62	0,101	0,46	0,44	0,7957	8,91***	2,39	0,237
29,0	322	14	20,06	0,077						
	371	17	20,42	0,081						
	413	19	19,36	0,099						
	Σ	50	19,92	0,087	0,44	0,42	0,8218	9,99***	2,24	0,257
	$\Sigma\Sigma$	200								

nucleolar size in the statistical calculations the volume of the nucleolus expressed as the portion of the total nuclear volume was chosen as variate value x. In order to get statistically comparable values the diameters of the nucleus (k) and nucleolus (n) were measured in all cells that were cut by a straight line. This line was drawn in a central longitudinal section perpendicularly to the direction of the length of the root and at a certain distance from the root tip. In those cases where the nucleus or nucleolus was not of circular shape in the microscope, an average diameter was determined. Now the relative size of the

TABLE 2. *Analysis of variance. Influence of temperature on the relative size of the nucleolus.*

	Degrees of freedom	Sum of squares	Mean square	Row No.	Quotient of variance (v^2)
Between temperatures	3	0,184656	0,061552	I	$v^2\left(\frac{\text{I}}{\text{II}}\right) = 62{,}30$
Within temperatures	196	0,193592	0,000988	II	$v^2\left(\frac{\text{I}}{\text{III}}\right) = 23{,}99$
Between roots	8	0,020531	0,002566	III	$v^2\left(\frac{\text{III}}{\text{IV}}\right) = 2{,}79$
Within roots	188	0,173061	0,000921	IV	$P \sim 0{,}01$
Total	199	0,378248			

nucleolus could be expressed as $x = n^3/k^3$. From Fig. 1 and Table 1, which contains the mean values of 3 roots in each of 4 given temperatures, it will be seen that the size of the nucleolus decreases at a rise of temperature. The statistical treatment of the material consisted in hierarchically classifying it in accordance with the directions given by BONNIER and TEDIN (1940). The quotient of variance v^2(I/III) in Table 2 indicates with great certainty that a considerable part of the total variation is due to the influence of the temperature. The variation between roots of one and the same temperature was less pronounced. [v^2(III/IV) shows that the probability is slightly less than 0,01 that the variation is a mere chance.]

The necessity of using a relative value for the nucleolar size is shown in Table 1, column 4, which contains mean values of the nuclear diameters (k). A statistical analysis of these values indicates a highly significant variation between roots within temperatures ($P < 0{,}001$), as well as a less significant variation between temperatures ($P \sim 0{,}01$).

Probably this variation between roots is caused by the fact that non-corresponding parts of the meristems were measured, the meristems being of different absolute size.

Table 1 also shows that it is correct to use $x = n^3/k^3$ (i. e. a function of n/k). The values in the columns 6—9 indicate that there is a close correlation between n and k. The t values in column 9 express a high degree of significance when compared with BONNIER and TEDIN's Table II (p. 322). From the conformity of the coefficients of the regression (col. 7) and n/k (col. 6) it may be deduced that the regression

TABLE 3. *Influence of temperature on the frequency of persisting nucleoli. Experiment data and analysis of covariance.*

Temp. °C	No. of roots with persisting nucleoli at metaphase per 24	No. of cells with persisting nucleoli			
		at metaphase		at metakinesis	
		per 10	total, per cent	per 10	total, per cent
11,5	22	4; 7	55	10; 10	100
16,5	12	1; 3; 7; 3	35	4; 8; 10; 10	80
23,5	3	0; 0; 0	0	0; 2; 4	20
29,0	0	0; 0; 0	0	0; 3; 2	16
r	— 0,7535	— 0,5217		— 0,6825	
t	11,11 ***	6,64 ***		10,14 ***	

line is straight and that its continuation runs through the point ($n = 0$; $k = 0$). The demonstration of this regression was considered necessary because BRETSCHNEIDER and HIRSCH (1937), in an investigation of the eggs of *Lima hians*, found the quantities to be rather independent.

Conclusion: *The diameters (and the volumes) of the nucleus and nucleolus are intimately correlated. The regression line is straight and runs through the origin. The part of the nucleus which is filled up by the nucleolus decreases at an elevation of temperature within the range 10°—30° C.*

3. *The dissolution of the nucleolus during prophase.* — The question whether temperature has an influence on the speed at which the nucleolus dissolves at the beginning of mitosis was clarified by two analyses. In the first one the number of roots was determined which in 4—5 central sections presented nucleoli at metaphase; 24 roots were investigated in this manner at each temperature. Table 3, column 2, shows that the number of persisting nucleoli at the metaphase stage decreases at an increase of temperature. A correlation factor r was determined with temperature as the independent variable, and absence

($y = 0$) or presence ($y = 1$) of persisting nucleoli as the dependent variable. That $r \neq 0$, i. e. really indicates a correlation, is borne out by a comparison of $t = \sqrt{\frac{(N-2)\, r^2}{1-r^2}}$ with the corresponding values of BONNIER and TEDIN's Table II. ($P < 0{,}001$.)

The second analysis in the latter part of the Table 3 likewise demonstrates an influence of temperature. In this analysis it was decided whether the nucleolus or remainders of it were still present in 10 metakineses and 10 metaphases in each of 2—4 roots at each temperature. In both stages of division the frequency of nucleoli decreases at an increase of temperature (significant r values). For reasons which are easily understood the frequency of nucleoli is greatest in metakinesis.

Conclusion: *At metakinesis and metaphase persisting nucleoli are more frequent at lower temperatures than at higher ones.*

DISCUSSION.

1. *The nucleolus as a coacervate.* — The results of the present investigation seem to indicate that the nucleolus is a separated phase out of a saturated solution: as a rule the solubility of a substance increases with an increase in temperature. Therefrom it follows that the volume of a crystal or separated phase which is in equilibrium with its saturated solution diminishes with a rise in temperature (an analogy to the size variation of the nucleolus). Further, on dilution, the crystal or separated phase is less rapidly dissolved at lower temperatures than at higher ones. (An analogy to the dissolution of the nucleolus; the disappearing of the nuclear membrane means dilution of the nuclear sap.) However, comparisons with the solubility conditions of low-molecular substances cannot be made without some restrictions (cf., however, BUNGENBERG DE JONG, 1938), for the solutes of the cell are to a large extent made up of high molecular substances, such as proteins and nucleotides, the solubility conditions of which are not yet fully clarified. These high-molecular substances constitute the dispersoid phase of a sol, and as a rule form a gel when precipitated. Under certain circumstances, however, the precipitation of a sol takes place as a de-mixing (coacervation; cf. BUNGENBERG DE JONG, 1932, 1936) into two liquid layers, the one being rich in colloids (coacervate) and the other poor in colloids (equilibrium liquid). The colloid particles of a coacervate attract one another by electrostatic (BUNGENBERG DE JONG) and VAN DER WAAL's forces in a wide sense (especially dispersion forces) (cf. WOLF, 1943, p. 465). In the first case, the colloid may

either be unitary with part of the particles recharged (autocomplex coacervation), or it may be made up of differently charged colloids and ions (complex coacervation). Antagonistic to the attraction a repulsion factor is at work, which is connected with hydratation of the colloids.

The influence of temperature on the complex coacervation of gelatine and gum arabic has been studied by BUNGENBERG DE JONG, HOSKAM and V. D. BRANDHOF-SCHAEGEN (1941). They found, among other things, that when temperature is raised the coacervate volume decreases slightly, and the solubility of the coacervate, measured as the dry weight of the equilibrium liquid, slightly increases. It is further known that the presence of neutral salts has an influence on coacervation. If the increase in the neutral salt concentration exceeds a certain value (this value may frequently be 0), the solubility of a coacervate increases (cf. BUNGENBERG DE JONG, 1932, 1936, 1937; BANK, 1941).

According to CASPERSSON's (1940) ultraviolet spectroscopic investigations of the egg cell of the sea-urchin and rabbit, the nucleolus contains proteins of the histone-absorption type (i. e. proteins rich in diamino-acids) in a very high concentration, as well as ribose nucleotides. The nucleus, with the exception of the nucleolus, contains proteins of the histone-absorption type as well as of the globulin-albumin (or intermediate) type, both in low concentrations. Measurements on the embryons of fowl gave the same results and showed the presence of basic proteins and ribose nucleotides in the cytoplasm (CASPERSSON and THORELL, 1941).

Observations of CASPERSSON and his co-workers on the distribution of basic proteins in the nucleus seem to support the hypothesis advanced earlier that the nucleolus was a coacervate (BUNGENBERG DE JONG, 1932, mentions the nucleolus, and FREY-WYSSLING, 1938, agrees with this hypothesis). The basic proteins are the chief coacervating colloid. Further evidence in support of this hypothesis is afforded by the results of the present investigation. The influence of temperature on the solubility of a coacervate and on the nucleolus agree. Probably the cytoplasm contains smaller quantities of the nucleolar constituents in question than the nuclear sap. If this assumption holds good, it will be easily understood why the nucleolus is dissolved on disappearance of the nuclear membrane (perhaps subsequent to an increase in permeability); further, the dissolution proceeds slower as the solubility decreases. The morphology of the nucleolus, too, speaks for its coacervate nature: Its globular shape and the absence of discontinuities as well as the mode of deformation of the persisting

nucleolus by the spindle suggest that it is a drop. Under the influence of the equatorial plate of the spindle, where the pressure on the nucleolus seems to be largest, it becomes either dumb-bell-shaped, and is then divided into two parts which often are of unequal size (no internal structure), or pear-shaped with the narrow end oriented in the equatorial plate. The nucleolus, or the parts of the divided nucleolus, are then ejected towards the spindle poles.

Several investigations have shown that on treatment of the cell with hypertonic neutral salt solutions, the nucleus grows optically homogeneous, and the nucleolus disappears (cf. BANK, 1941, and STRUGGER's fig. on p. 29, 1932). Probably this phenomenon is closely connected with the neutralizing effect of crystalloid ions on coacervation.

2. *The function of the nucleolus.* — CASPERSSON (e. g. 1941) supposes that the histone-like proteins, which are the chief constituents of the nucleolus, are produced by the heterochromatic parts of the chromosomes. He also suggests that these lower proteins are consumed at the synthesis of nucleotides and proteins in the cytoplasm. Previous to this process nucleolar substance would have to diffuse from the nucleolus (or the chromosomes) to the nuclear membrane and out into the cytoplasm. A fall of concentration of nucleolar substance from the nuclear sap to the cytoplasm is necessary for such a diffusion stream, which is in agreement with the observations on the solubility conditions of the nucleolus in the present paper. From the colloidal standpoint (cf. BUNGENBERG DE JONG's exposition, 1937 b) the nuclear sap is a saturated solution of histone-like proteins, and the dissolution of the nucleolus in the cytoplasm implies that in this part of the cell the concentration of nucleolar substance is lower.

Another possible explanation of the variation in behaviour and size of the nucleolus must be taken into consideration. The total quantity of nucleolar substance existing in a nucleus at a certain moment is dependent on two complicated chemical reactions, viz. on a process of formation and on a process of consumption. In normal cell development these two reaction complexes are balanced. Otherwise a deleterious excess or deficit of nucleolar substance would arise. This might be demonstrated in the root meristems of *Salix*, where the duration of the resting stage is very long (= few mitoses). Now, the temperature-conditioned variation of the size of the nucleolus might be explained by the fact that the speeds at which histone-like proteins are produced and consumed show a different dependence on changes in temperature. If this were true, the size of the nucleolus would continue to increase at a

temperature above (below) the optimal one and (if at all formed) it would decrease at a temperature below (above) the optimal one during the duration of the resting stage; that is to say, the variation of x would be different at different temperatures. Table 1, column 11, shows, however, that the relative variation of x expressed as $\frac{\text{standard deviation } (s)}{\text{mean value of } x\ (\bar{x})}$ is as good as constant. Therefore, it may be assumed that formation and consumption of nucleolar substance balance each other at the temperatures in question.

At the beginning of mitosis the chromosomes discontinue protein production, and, presumably, the synthesis of proteins and nucleotides in the cytoplasm is likewise arrested. It is natural that a mutual variation between the moments at which these reactions cease, and a variation of the speeds at which the retardations occur, cause changes in the point at which the nucleolus disappears at prophase. Undoubtedly, such variations in time play an important rôle in those cases where the persistence of the nucleolus is genetically controlled. HÅKANSSON and LEVAN (1942) show a high frequency of persisting nucleoli in *Pisum*; as is the case within the genus *Salix*, genetical factors, no doubt, are at work here, since LEVAN (unpubl.) found that such deviating nucleoli are very common in several other genera of Leguminosae. Concerning *Pisum* there are facts indicating a continued protein production of the heterochromatin during prophase, when it should normally have ceased (nucleolar pieces on the chromosomes; cf. point 3 below). Probably, in *Pisum* the dissolution of the nucleolus is also retarded by the fact that the cytoplasm (at least in meiosis) seems to be almost saturated with nucleolar constituents. Extranuclear nucleoli formed at anaphase I or telophase I grow during interkinesis. And even if HÅKANSSON and LEVAN's observations on the nucleolar conditions at meiosis do not unreservedly hold good for mitosis they support the assumption that a heterochromatin activity extended to the division stages is one of the causes of nucleolar persistence.

In this connection it should be mentioned that the solubility of the substances which (besides water) make up the main part of the nucleolus is strongly controlled by the pH and ion activity in the nuclear sap as well as by the composition of the nucleolus itself (varying »strength» of the complex coacervation). All these factors may be conditioned by temperature as well as genetically controlled. (As to the variation in nucleolar composition, see SCHULTZ, CASPERSSON and AQUILONIUS, 1940; SERRA, 1942, p. 121.)

The causes of the variations of the nucleolus which have been investigated or discussed here may be summed up as follows:

(I) Variation of solubility, which is
  (A) directly temperature-conditioned,
  (B) dependent on variation of the pH, ion activity, and the composition of nuclear sap and cytoplasm as a whole.
    (a) This variation is partly temperature-conditioned,
    (b) partly dependent on other physiological factors.

(II) Variation of the speed and duration of different reaction complexes in the cell, in the first instance formation and consumption of nucleolar substance as well as the progress of prophase.
  (A) This variation is partly temperature-conditioned,
  (B) partly dependent on other physiological factors.

The cause of variation expressed here as »other physiological factors» consists partly of a genetically controlled variation, partly of a variation between cells having different locations in the root, and finally of a variation the causes of which are undefined.

A large number of observations on persisting nucleoli have been published in the cytological literature (cf. DARLINGTON, 1937, p. 306; TISCHLER, 1942, p. 200). The discrepancy of these observations on a material which was cultivated under conditions that were either uncontrolled or not fully described makes TISCHLER (l. c., p. 201) attribute a »launischen Charakter» to the nucleolar persistence. Probably, a closer study would reveal basic features in accordance with the above schedule.

3. *The formation of the nucleolus.* — A protein solution is readily supersaturated (cf. PAULI and VALKÓ, 1933, p. 101). If the assumption holds good that the nucleolus is a separated coacervate phase, then every structure in the nucleus of suitable constitution will serve as a condensation centre at precipitation (coacervation). Nucleoli, as a rule, are formed around the SAT-filaments in plants, where these are distinguishable (HEITZ, 1931; TISCHLER, 1942, p. 205). Evidently, these are superior condensation centres [perhaps in connection with the often described heterochromatic character of the satellites (cf. TISCHLER)]. At the high hydrogen-ion activity of the meristem (cf. EHRENBERG, 1946) the basic proteins are positively charged, and therefore structures of similar chemical constitution as well as substances of opposite electrical charge may act as crystallization centres; (in the latter complex coacervation the »higher» proteins and nucleotides probably participate as well as crystalloid ions). If a chromocentre develops (cf. CASPERSSON

and THORELL, 1941), it might play the same rôle as the SAT-filaments. The correspondence of chromocentres to satellites is often obvious (HEITZ, 1932; TISCHLER, l. c., p. 176). If the cell should lose the normal condensation centres for nucleolar substance, the latter will find new ones on existing structures as soon as the nuclear sap gets saturated. A good example of this is *Allium amplectens*, which, in contradistinction to related species, is without satellites, and therefore every cell forms primarily 10—12 small nucleoli, which later fuse, and which seem to be attached to several chromosomes (LEVAN, 1940). The species with satellites form a corresponding number of larger nucleoli.

4. *Nucleolus and mitosis.* — In the persisting nucleolus nature has given us a sort of model body inside the spindle. Though changes in surface tension might explain the exit of the nucleolar remnants out of the spindle, the deformation of the spindle seems to indicate a mechanical pressure: the potential energy is greatest when the nucleolus is in the equatorial plate. Most likely, these mechanical forces partly also account for the larger chromosomes lying on the periphery of the metaphase plate (cf. DARLINGTON, l. c., p. 524). Further, the nucleolar pieces often leave the spindle during metakinesis, i. e. at the same time as the chromosomes move towards the equatorial plate. This indicates that the chromosomes interfere with the spindle by chemical forces. The nucleolar fragments which are not attached to the spindle move to that part of the cell where their potential energy is least, whereas the movement of the chromosomes towards the equatorial plate is an endothermic process.

The nucleolar rests always move in the direction of the spindle fibres. This indicates that the spindle is mechanically anisotropic, in addition to the optical anisotropy shown by SCHMIDT (1939) and others.

Finally, the nucleolar persistence definitely refutes the old hypothesis that the spindle is built up directly of nucleolar substance (TISCHLER, l. c., p. 202). In some cases the size of the nucleolus is not perceptibly decreased at metaphase, though the spindle is simultaneously fully developed.

June 27, 1945.

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ABERRANT Y CHROMOSOMES AND SEX EXPRESSION IN MELANDRIUM ALBUM

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I. INTRODUCTION.

IN a previous publication, the present author has described an intersexual *Melandrium album* plant designated S. 415—1 (WESTERGAARD, 1940, pp. 68—69, Figs. 27 *b* and 29). Apparently, this plant was an *XXXX* ♀, however, the behaviour of the sex chromosomes during meiosis indicated that the one was probably a fragmented *Y* chromosome, since a ring of four sex chromosomes, to be expected in an *XXXX* plant, was never observed. Definite evidence is now available that this is actually the case, since it has been possible in root-tip mitoses to identify the fragmented *Y* chromosome from the position of the centromere. Later, the same type appeared twice in the offspring of triploid *Melandrium*, and the genetical and cytological significance of the aberrant chromosome, from now referred to as the Y^1 chromosome, has been studied more thoroughly. In the same material of triploid offspring plants two new structural changes of the *Y* chromosome have been detected, which are denominated the Y^2 and Y^3 chromosomes. The Y^2 type, which has arisen twice, is probably the result of a translocation between the differential arms of *X* and *Y*, whereas the origin of Y^3 is due to a terminal fragmentation in the homologous arm of *Y*. A brief account of these types has been published recently (WESTERGAARD, 1946); the present publication gives a more detailed description of the material.

II. GENETICAL SECTION.

The Y^1 type. — The first aberrant plant (S. 415—1) appeared in F_2 from a cross between experimentally raised tetraploids. The second plant (S. 443—9), which had the chromosome constitution $2n = 43 + + XXXY^1$, derived from a crossing between triploids ($33 + XXX$ ♀ $\times \times 33 + XXY$ ♂). The third plant (S. 599—118) with the chromosome number $2n = 44 + XXXY^1$, appeared in an F_3 generation resulting from

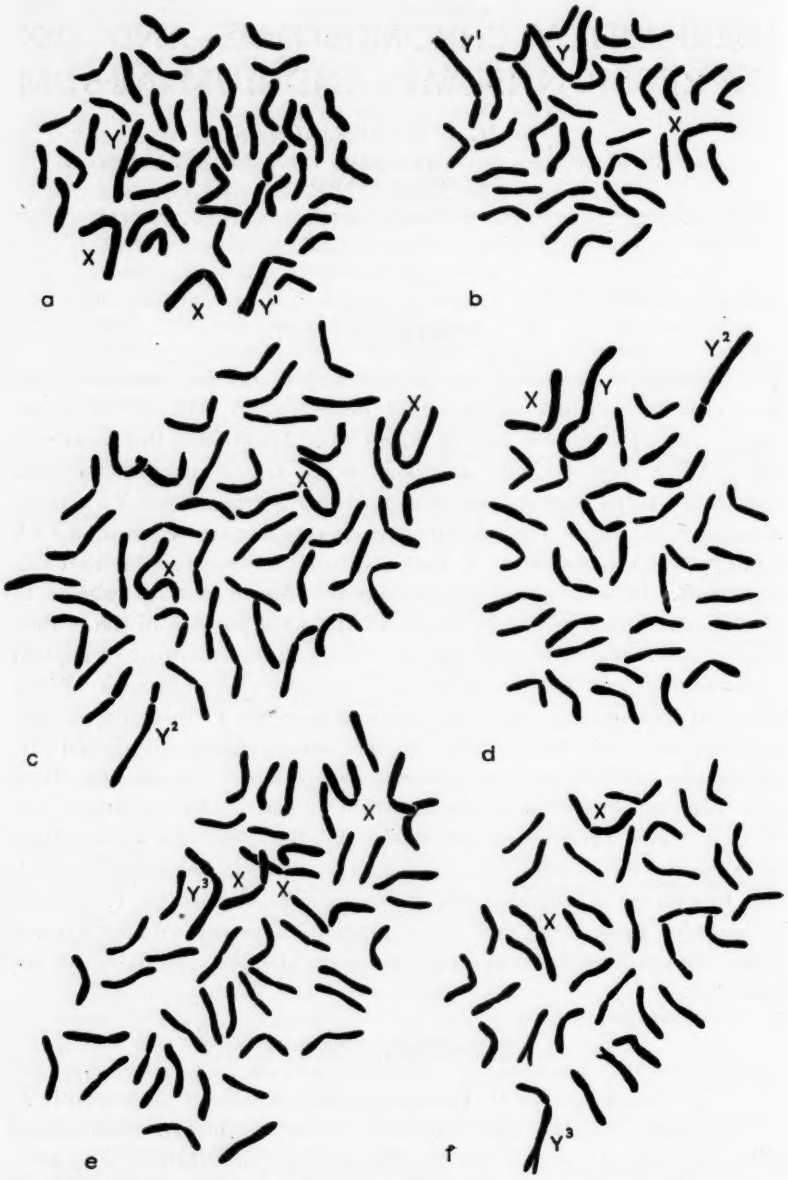


Fig. 1. Mitotic metaphases from plants with aberrant *Y* chromosomes. *a*: 43 + + *XXY*^{1}*Y*^{1}. *b*: 33 + *XYY*^{1}. *c*: 40 + *XXXY*^{2}. *d*: 33 + *XYY*^{2}. *e*: 44 + *XXXY*^{3}. *f*: 32 + *XXY*^{3}. — Magnification 3500 ×.

two generations of selfing triploid intersexual plants with normal sex chromosomes (*XXXY*).

The three plants looked normal; they were fairly robust and did not deviate morphologically from other polyploid *Melandrium*. They were strictly intersexual in all the flowers during the whole growing period (cf. WESTERGAARD, 1940, Fig. 31 *b*, pp. 16—17, and Plate I *a* of the present publication). They correspond to the »Euhermaphroditen» of CORRENS, whereas most of the other triploid *Melandrium* intersexes with normal sex chromosomes are typical »Androhermaphroditen» and pure males at the end of the growing period.

Fig. 2. Sex chromosomes from 11 plants with aberrant *Y* chromosomes, each figure deriving from a single cell. *d*: $XXXY^1$. *b*: XXY^1Y^1. *c*: $XXYY^1$. *d*: $XY^1Y^1Y^1$. *e*: $XXXY^2$. *f*: XXY^2Y^2. *g*: XXY^2. *h*: XYY^2. *i*: $XXXY^3$. *j*: XXY^3. *k*: XYY^3. — Magnification 3500 ×.

Cytologically, the three $XXXY^1$ intersexes are characterized by their abnormal *Y* chromosome which is slightly longer than the *X* and has a submedian centromere (Figs. 1 *a*—*b*, 2 *a*—*d*, and Plate II *c*). The long arm corresponds to the arms of the normal isobrachial *Y* chromosome, whereas the short arm is slightly more than $^1/_3$ that of the long arm and considerably shorter than the shortest arm of the normal *X* chromosome. After having gained some experience we can identify Y^1 with great certainty in the somatic metaphases. Regarding meiosis, the absence of the tetravalent associations, the formation of which requires a chiasma formation between Y^1 and the differential arms of *X*, is the most important feature (for details, see next section). Otherwise, in size Y^1 resembles a normal *X* chromosome during meiosis and, under favour-

able conditions only, it can be identified from the fact that its total length is slightly longer than *X* (cf. Fig. 3).

The offspring of the first three *XXXY*^{1} plants have been traced for several generations, the genetical results being the following:

By selfing S. 415—1, 11 ♀♀ were produced, one of which (S. 445—13) yielded an F_2 generation comprising 22 ♀♀ and 50 ♀♀. The cytology of these plants has not been studied. One of the F_2 intersexes was pollinated with a tetraploid *XXXY* ♂. The cross results in 2 ♀♀ : 3 ♀♀ : : 6 ♂♂. The chromosome constitution of the females was not studied. Two of the ♀♀ had the sex chromosome constitution *XXXY*^{1}, the third was an *XXXXY*^{1} plant. The males consisted of 1 *XXXY*, 3 *XXYY*^{1}, and 1 *XXXYY*^{1} plants. Supposing that the constitution of the female parent was *XXXY*^{1}, the cross *XXXY*^{1} × *XXXY* should give rise to *XXXX* ♀♀, *XXXY*^{1} ♀♀, *XXYY*^{1} ♂♂ and *XXXY* ♂♂ in equal proportions. The appearance of the *XXXXY*^{1} and *XXXYY*^{1} plants may be due either to non-disjunction or the constitution of the intersexual female parent may actually have been *XXXXY*^{1}.

The second *Y*^{1} plant (S. 443—9) produced 51 ♀♀ by selfing (S. 537). The somatic chromosomes were counted in 49 of these plants; the result is grouped in Table 1.

TABLE 1. *S. 537.*

Sex chromosomes	Autosomes							Total
	40	41	42	43	44	45	46	
XXXY^1	1	1	4	12	6	4		28
XXY^1*Y*^{1}	1	2	1	8	3	1		16
XXY^1				1	1		1	3
XXXXY^1	1			1				2
Total	3	3	5	22	10	5	1	49

As is to be expected in the offspring of a triploid, these plants showed some morphological variation. However, although some variation was found in the development of the anthers and gynoecia (cf. Plate I *a*), no correlation could be detected between this variation and the sex chromosome constitution, e. g., no morphological or sexual difference was found between the *XXXY*^{1} and *XXY*^{1}*Y*^{1} plants. This agrees with the fact that the tetraploid *XXXY* and *XXYY* males are indistinguishable (cf. WESTERGAARD, 1940). Hence, the variation must be due to the varying number of autosomes, only.

Table 1 reveals that there is only a slight variation in the autosome numbers of the 49 plants, the maximum being very close to the tetra-

ploid number 2n = 44. This phenomenon was observed in all triploid *Melandrium* intersex plants which tend to stabilize themselves on the tetraploid chromosome level and not on the diploid level, as is generally the case in other triploid plant materials (WESTERGAARD, unpublished).

Regarding the sex chromosomes, the material grouped in Table 1 deviates significantly from the ratio which is to be expected from selfing an $XXXY^1$ intersex, viz. 1 $XXXX$ ♀ : 2 $XXXY^1$ ♂♀ : 1 XXY^1Y^1 ♂ ($\chi^2 = 13{,}90$, $P < 0{,}001$). The absence of ♀♀, which was also found in the offspring of S. 415—1, is a peculiar phenomenon. In some of the other sowings we even meet with a great deficit of females. This may be due to certation, since deviating sex ratios are often found in *Melandrium* (cf. CORRENS, 1928); in the normal diploid *Melandrium*, however, the situation is reverse, since the females generally occur in excess. However, the competition between the XX carrying and the XY^1 carrying pollen grains in polyploid *Melandrium* may very well be different from that occurring between normal X and Y pollen in the diploid strains. Another explanation may be that nuclei containing XY^1 have a greater chance of forming the megaspores than those containing XX. Some evidence will be given later that a competition actually may occur between the four haploid cells, resulting from the meiosis in the megaspore mother cells.

Four of the intersexual plants of S. 537 were selfed and yielded the following offsprings: S. 537—1 ($2n = 45 + XXXY^1$) gave rise to 6 ♀♀, 12 ♂♀, and 76 rosettes. S. 537—2 ($2n = 40 + XXXY^1$) produced 32 ♀♀, 79 ♂♀, 315 rosettes. 537—21 ($2n = 42 + XXY^1Y^1$) yielded 2 ♀♀, 28 ♂♀, and 12 rosettes. The fourth plant, 537—49 ($2n = 43 + XXXY^1$), yielded 12 ♂♀ and 8 rosettes. Unfortunately, that year the number of rosettes which, for technical reasons, could not be kept through the winter, was fairly high, the plants being sown very late, because of a very hard and long winter.

Among the above sowings the offspring of the XXY^1Y^1 plant (S. 537—21) is of special interest. The meiotic behaviour of the XXY^1Y^1 plants resembles that of the $XXYY$ males (cf. WESTERGAARD, 1940). Therefore, a great excess of XY^1 gametes should be produced and, by selfing, most of the offspring is expected to be XXY^1Y^1 intersexes. In addition, a small number of XX and Y^1Y^1 gametes will be formed and, therefore, a small proportion of $XXXX$, $XXXY^1$, and $XY^1Y^1Y^1$ plants are to be expected. Unfortunately, the chromosome constitution was only studied on the two female plants and on one intersex with a somewhat deviating habit, viz. a weak, narrow-leaved plant the sex expression of

which, however, did not deviate from the other intersexes. Both the females had four normal *X* chromosomes while the intersex was an $XY^1Y^1Y^1$ plant (Fig. 2 *d*).

Two of the intersexual offspring plants of S. 537—2 [sowing numbers S. 602—105 ($2n = 44 + XXXY^1$) and S. 602—20 ($2n = 41 + + XXXY^1$)] were selfed and yielded an F_2 generation. The first plant produced 9 ♀♀ and 17 ♂♀, the second one 8 ♀♀ and 20 ♂♀. The chromosome constitution was determined in 25 plants from these sowings; the result is collected in Table 2.

TABLE 2. *S. 712 + 713.*

Sex	Sex chromosomes	Autosomes 39	40	41	42	43	44	45	46	Total
♀♀	*XXX*					2				2
	XXXX	1				1	3	2		7
♂♂	XXY^1		1							1
	$XXXY^1$		1	1	1	1	2	1	2	9
	$XXXXY^1$		1							1
	XXY^1Y^1					1	2	1		4
	$XXXY^1Y^1$			1						1
	Total	1	3	2	1	5	7	4	2	25

The third intersexual Y^1 plant (S. 599—118) appeared in an F_2 generation from selfing triploid intersexes. By selfing, the plant produced 10 ♀♀ and 19 ♂♀, hence, the deficit of females, observed in the offspring of the two other Y^1 plants, was lacking in this case. The chromosome constifution of some of the plants is given in Table 3.

TABLE 3. *S. 708.*

Sex	Sex chromosomes	Autosomes 42	43	44	45	Total
♀♀	*XXX*		1			1
	XXXX	2 *	3 **	1		6
♂♂	$XXXY^1$	1		1	2	4
	XXY^1Y^1			2		2
	Total	3	4	4	2	13

* 1 plant with fragment. ** 2 plants with fragments.

Crossings between Y^1 ♂♀ and diploids. — The intersexual plant S. 599—118 was pollinated with a normal diploid male plant and from this crossing 11 ♀♀, 20 ♂♀, and 24 ♂♂ were raised (cf. Plate I *e*). The

expected ratio is 1 *XXX* ♀ : 1 *XXY*^{1} ♀♂ : 1 *XXY* ♂ : 1 *XYY*^{1} ♂. The observed frequencies deviate somewhat from the expected ratio 1 ♀ : : 1 ♀♂ : 2 ♂♂ ($\chi^2 = 3{,}84$, $P < 0{,}2$), because the proportion of females is too low again.

The somatic chromosomes were counted in 24 plants which are given in Table 4.

TABLE 4. *XXXY*^{1} ♀♂ × *XY* ♂.

Sex	Sex chromosomes	Autosomes 30	31	32	33	34	35	36	Total
♀♀	*XX*				3				3
	XXX	1		1					2
♀♂	*XY*^{1}						1	1	2
	XXY^1			3	4				7
♂♂	*XXY*			1	4				5
	XYY^1			2 *	2	1			5
	Total	1		7	13	1	1	1	24

* 1 plant with a fragment.

The 5 *XX* and *XY*^{1} plants must be due to non-disjunction, but the proportion is remarkably high. The variation in the number of autosomes indicates the triploid origin of the female parent, although it seems that the plant is almost stabilized on the tetraploid chromosome number.

Crossings between different Y^1 *strains.* — The intersexual plant S. 599—118 was pollinated with an *XXXY*^{1} intersex which derived from S. 443—9. The crossing resulted in 5 ♀♀ and 54 ♀♂♀♂ (theoretically $14{,}75 : 44{,}25$, $\chi^2 = 8{,}58$, $P < 0{,}01$). Hence, a deficit of females is found even in this sowing. The chromosome constitution has been worked out in the 23 plants which are grouped in Table 5.

TABLE 5. *599—118* × *443—9.*

Sex	Sex chromosomes	Autosomes 38	39	40	41	42	43	44	45	46	47	Total
♀♀	*XXX*							1				1
	XXXX							1				1
♀♂	*XXY*^{1}						1					1
	XXXY^1	1			4	1	1	3 *	1	2	1	14
	XXY^1*Y*^{1}					4 **		1	1			6
	Total	1			4	5	2	6	2	2	1	23

* 1 plant with a fragment. ** 2 plants with fragments.

The meiotic behaviour of the XXY^1Y^1 plants found in this crossing is identical with that of the XXY^1Y^1 plants produced by selfing, e. g., the Y^1 chromosomes occasionally form chiasmata in the short arms (for details, see next section). This shows that the Y^1 chromosomes of the plants 599—118 and 443—9 are identical, hence the Y chromosome has been broken twice at the same point. The first Y^1 type (415—1) has not been crossed with the two other types. However, the length of the short arm seems to be exactly equal in the three plants, and it is therefore most likely that the Y chromosome has undergone three independent, but identical fragmentations in this polyploid *Melandrium* material.

The above analysis of the genetical behaviour of the Y^1 types proves definitely that the intersexual condition of the plants depends upon the presence of the Y^1 chromosome.

The Y^2 type. — The first Y^2 plant (S. 536—21, $2n = 41 + XXXY^2$) appeared in an F_2 generation raised from selfing a triploid intersexual plant. The parental plant of the Y^1 intersex 599—118 was a sister plant to S. 536—21. The plant was a typical »euhermaphrodite», both the anthers and the gynoecia being well developed in all flowers (Plate I *b*). The flowers looked very much like those containing the Y^1 chromosomes. However, meiosis in the $XXXY^2$ plants was remarkably different from that of the $XXXY^1$ plants. In the former, a high proportion of configurations requiring an exchange of chiasmata between the short arm of Y^2 and the differential arm of X was observed (for further details, see the cytological section), these types being totally absent in the $XXXY^1$ plants. Therefore, the Y^2 chromosome behaves exactly like an X chromosome during meiosis and, in fact, the type was first considered an $XXXX$ ♀. However, a closer examination of the somatic chromosomes showed that one of the chromosomes actually was an aberrant Y chromosome composed of a long arm corresponding to the arm of the normal Y and a very short arm, about $^1/_4$ the length of the other arm, and considerably shorter than the short arm of Y^1 (Figs. 1 *c—d*, 2 *e—h*, Plate II *b*). The whole chromosome was slightly shorter than X, but generally the difference is not visible during meiosis. As will be discussed later, it is most probable that Y^2 is the result of a translocation between a short terminal segment of the differential arm of X and the whole differential arm of Y.

The second Y^2 plant appeared in a sowing obtained from four generations of selfing triploid intersexual plants. The chromosome number of the second plant (S. 607—5) was likewise $2n = 41 + XXXY^2$.

The development of the male and female organs in the two plants was almost the same.

By selfing the first plant, 110 ♀♀ and 204 ♂♂ were raised. The ratio to be expected is 78,5 : 235,5, and the deviation from expectation was highly significant ($\chi^2 = 16{,}85$, $P < 0{,}001$), e. g., in this case a great excess of females was produced, in contradistinction to the conditions found in the Y^1 type. From the second plant, 78 ♀♀ and 199 ♂♂ were secured. The deviation from expectation was non-significant in this case (exp. ratio: 69,25 : 207,75, $\chi^2 = 1{,}47$, $P < 0{,}3$).

The chromosome constitution has been worked out in 34 plants derived from S. 536—21 (Table 6). The table reveals that the intersexual condition depends upon the presence of the Y^2 chromosome. The variation in the number of autosomes agrees with that observed in the Y^1 pedigrees. Also in this material, the sex expression in the different Y^2 plants shows some variation (cf. Plate I *b*—*c*), but no strict male or female flowers have been detected on the intersexual plants. Again the variation must be ascribed to the varying number of autosomes; no difference was to be found in the sex expression of the XXY^2Y^2 and $XXXY^2$ intersexes.

TABLE 6. *S. 600, from selfing S. 536—21.*

Sex	Sex chromosomes	Autosomes 39	40	41	42	43	44	45	Total
♀♀	$XXXX$		2	2 *	3	1			8
♂♂	$XXXY^2$	2	2	4 *	6	2	2	1	19
	XXY^2Y^2			1	1	5			7
	Total	2	4	7	10	8	2	1	34

* One plant with a fragment.

Two of the intersexual plants from S. 600 were selfed and yielded a second generation. The first one (S. 600—280) was an $XXXY^2$ plant and gave rise to 59 ♀♀ and 157 ♂♂, a proportion not significantly different from the expected one, viz. 54 : 162 ($\chi^2 = 0{,}62$, $P < 0{,}5$). The second plant (S. 600—200) belonged to the XXY^2Y^2 type and produced 10 ♀♀ and 66 ♂♂. A great excess of XY^2 gametes is to be expected from an XXY^2Y^2 intersex, giving rise to a high proportion of intersexes by selfing. Although the expected frequency cannot be calculated with certainty, the proportion of females seems to be fairly high.

Crossing between Y^2 ♂♂ and diploids. — The XXY^2Y^2 plant S. 600—200 was pollinated with a diploid XY male. The crossing

resulted in 1 ♀, 15 ♀♀, and 18 ♂♂ (cf. Plate I *d*); the chromosome numbers were determined on 27 ♀♀ and ♂♂; the result is tabulated in Table 7.

TABLE 7. XXY^2Y^2 ♀ × XY ♂.

Sex	Sex chromosomes	Autosomes 30	31	32	33	Total
♀♀	XXY^2	1	2	2	4	9
♂♂	XXY	1	1	1		3
	XYY^2	1	1	10	3	15
	Total	3	4	13	7	27

In the reciprocal crossing, 2n ♀ × ♀, the $XXXY^2$ plant S. 600—280 was used as the pollen parent, the cross yielded 14 ♀♀ and 9 ♀♀, the chromosome constitution was the following (Table 8).

TABLE 8. XX ♀ × $XXXY^2$ ♀.

Sex	Sex chromosomes	Autosomes 31	32	33	34	35	Total
♀♀	XX			1			1
	XXX		4	7	1	1	13
♀♀	XXY^2	1	4	2	1		8
	Total	1	8	10	2	1	22

Supposing that the XXY^2Y^2 plants produce a high proportion of XY^2 gametes and a small number of XX and Y^2Y^2 gametes, pollination with X or Y pollen is expected to give a high proportion of XXY^2 ♀♀ and XYY^2 ♂♂ and, moreover, a small proportion of XXX ♀♀, XXY ♂♂ and a type with the constitution YY^2Y^2, the latter probably being inviable. The cross XX ♀ × $XXXY^2$ ♀ will give rise to XXX ♀♀ and XXY^2 ♀♀ in equal proportions.

The above described crossings prove definitely that the intersexual condition of the plants in the above sowings depends upon the presence of the Y^2 chromosome. As regards the homology of the short arms of the Y^2 chromosomes derived from S. 536—21 and 607—5, crossings between the two strains have given rise to XXY^2Y^2 plants in which the two aberrant Y chromosomes derive from different strains. Meiosis in such plants shows the same multivalent associations as observed in plants derived from selfing intersexes belonging to the same strain.

This proves that the ends of the short arms in the two strains are homologous.

The Y^3 type. — This type has appeared once only in this material. The first plant was found in S. 599 and was a sister plant to the Y^1 plant S. 599—118. The plant was slightly intersexual, but at the end of the growing period all the flowers were purely male, hence the plant must be classified as an »androhermaphrodite». The plant is, furthermore, characterized by its degenerative anthers and, therefore, it is male sterile. An investigation of meiosis showed four sex chromosomes, one of these, however, was always found to be univalent (cf. Fig. 3 *c*). In the somatic mitosis, 44 autosomes, 3 normal *X* chromosomes, and one aberrant *Y* chromosome were counted. The Y^3 chromosome is longer than the two other types, and longer than *X*. It has a submedian centromere, the long arm corresponding to the arm of a normal *Y* chromosome. The short arm is about half the length of the other arm, and a constriction is sometimes seen at about the middle (Figs. 1 *e—f*, 2 *i—k*, Plate II *d*), this constriction is occasionally observed also in the normal *Y* chromosomes.

The first Y^3 plant being male sterile, the type could only be propagated via the female side. From pollination with a normal diploid *XY* ♂, an offspring generation was raised consisting of 26 ♀♀, 34 ♂♂ with normal anthers, 10 ♂♂ with degenerative anthers (Plate I *f*), 1 ⚥ with normal anthers, and 2 ⚥⚥ with degenerative anthers like the female parent. The intersexual plants were all weak »androhermaphrodites», only the first flowers being slightly intersexual. The chromosome numbers were determined on root-tip mitoses on 35 of these plants, and the result is given in Table 9.

TABLE 9. $XXXY^3$ ⚥ × *XY* ♂.

Sex	Sex chromosomes	Autosomes 31	32	33	34	35	Total
♀♀	*XX*		2	1			3
	XXX	1	3	5	1	2	12
♂♂ sterile	XXY^3		1		3		4
	$XXXY^3$		1 *	3			4
♂♂ normal	*XY*			1		1	2
	XXY		4 **	1			5
	XXXY		1				1
	XYY^3	1	1	1			3
	$XXYY^3$	1					1
	Total	3	13	12	4	3	35

* Slightly intersexual. ** 1 plant slightly intersexual.

This crossing proves that the Y^3 chromosome is responsible for the degeneration of the anthers leading to pollen sterility, whereas it does not affect the sexual expression of the plants. The intersexuality of the original plant and of the three offspring plants is not due to the presence of Y^3, but must be ascribed to an accumulation of female genes in the autosomes.

Eight of the nine sex chromosome types which are found in Table 9 are to be expected from the cross $XXXY^3 \times XY$. The three X chromosomes will normally be separated in such a way that XX goes to one pole and X to the other pole. If the univalent Y^3 in half the cases joins the two X chromosomes and in half the cases the single X, four types of gametes will appear in equal proportions, viz. XXY^3—X—XX—XY^3; by fertilizing with X or Y pollen, the following eight combinations are possible: $XXXY^3$—$XXYY^3$—XX—XY—XXX—XXY—XXY^3—XYY^3. The appearance of the $XXXY$ plant must be due to non-disjunction, the three X chromosomes going to the same pole. The proportion females : males with normal anthers: males with degenerative anthers should be 1 : 2 : 1. The observed proportion 36 : 34 : 10 deviates significantly from this ratio ($\chi^2 = 6{,}70$, $P < 0{,}05$), the females occurring in excess and males with degenerative anthers in deficit. This may indicate that ovules containing the Y^3 chromosome are less viable than those containing only normal X chromosomes, e. g., the Y^3 chromosome which is absolutely lethal on the male side behaves as a sublethal on the female side.

III. THE BEHAVIOUR OF THE ABERRANT Y CHROMOSOMES DURING MEIOSIS.

The meiotic behaviour of the most important types has been studied in aceto-carmine after pre-fixation in Carnoy (without chloroform). MANTON's squash method (cf. WESTERGAARD, 1940) gave the best plates. The results are grouped in Tables 10 and 11, and some of the most critical types are pictured in Figs. 3 and 4, and in Plate II *e*—*i*. It should be kept in mind that, generally, the aberrant Y^1 and Y^2 chromosomes cannot be distinguished from the X chromosomes neither by their size nor by the position of the centromere. The associations drawn in Figs. 3 *a* and *e* and Fig. 4 *a* are selected types from unusually favourable plates.

The difference in the behaviour of the $XXXY^1$ and $XXXY^2$ associations in meiosis appears from Table 10. Y^1 behaves like an ordinary Y chromosome, the types and the frequencies of the associations being

almost equal to those described in the *XXXY* ♂♂ (WESTERGAARD, 1940, pp. 59—62; cf. Figs. 22, 34 and 35). Association No. 6, only, is somewhat more frequent in this material than the corresponding type in the *XXXY* material (cf. WESTERGAARD, 1940, Fig. 34, No. 43), whereas number 5 is less frequent than the corresponding type (No. 25) in the normal material. In the $XXXY^2$ plants the associations of the four sex chromosomes are strikingly different, the types Nos. 8—9—10 which

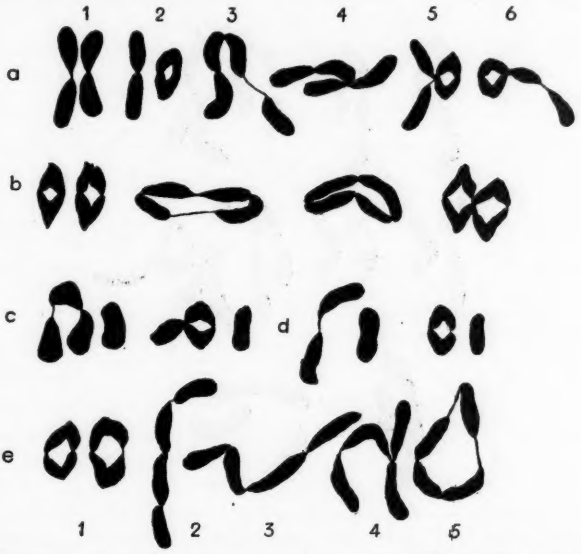


Fig. 3. Associations of the sex chromosomes in *a*: Six $XXXY^1$ cells in which the Y^1 chromosome can be identified. In *1*, *2*, *3* and *5* Y^1 is the lowest chromosome to the left, in *4* and *6*, Y^1 is the chromosome to the right. *b*: Four $XXXY^2$ associations in which the differential arms of Y^2 and *X* are associated. *c* and *d*: $XXXY^3$ and XXY^3 associations, showing the univalent Y^3 chromosome. *e*: XXY^1Y^1 associations showing chiasma formations in the differential arms of the Y^1 chromosomes. *2* is an $X—Y^1—Y^1—X$ chain, *3* an $X—X—Y^1—Y^1$ chain. In *4* the two *Y* chromosomes are the left ones. From aceto-carmine smears. — Magnification 2300 ×.

were not observed in the Y^1 plants occur in more than 60 per cent of the cells. These associations require a chiasma formation between the short arm of Y^2 and the differential arm of one of the *X* chromosomes. The actual chiasma frequency in this short arm is probably considerably higher than it appears from the frequencies of types 8—9 and 10, since types 2—7 very well may include, e. g., chains of the constitution $X—X—Y^2—X$. Generally, however, such a chain cannot be distinguished

from an X—X—X—Y^2 chain and, therefore, the exact chiasma frequency in the short arm of Y^2 cannot be calculated, the 60 per cent being a minimum estimate.

The sex chromosome associations have been studied in 80 XXY^1Y^1 plates (cf. Fig. 3 *e*). The most interesting result is the demonstration of a chiasma exchange between the short differential arms of the Y^1 chromosomes. This appears from the occurrence of type No. 8 (2 cases) and type No. 9 (3 cases). Moreover, it has sometimes been possible to

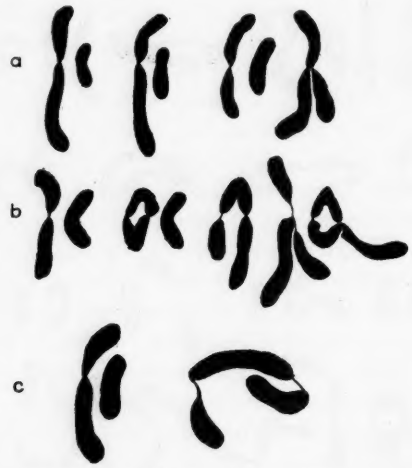


Fig. 4. Associations of the sex chromosomes in *a*: XYY^1. From the left: Y—Y^1 bivalent with univalent X; Y—X bivalent with univalent Y^1 (these types can generally not be separated); X—Y^1 bivalent with univalent Y and to the right the only trivalent type which is observed in these plants. *b*: XYY^2. From the left: X—Y^2 with univalent Y; X—Y^2 with univalent Y; Y^2—X—Y (generally not distinguishible from the X—Y^2—Y chains) and the two other trivalent types which are found in these plants. *c*: X—Y—Y^3, an X—Y bivalent with univalent Y^3 and an X—Y—Y^3 chain. From aceto-carmine smears. — Magnification 2300 ×.

identify the Y^1 chromosomes in the chains of four and, although the sequence Y^1—X—X—Y^1 seems to be by far the most frequent, the chains X—X—Y^1—Y^1 and X—Y^1—Y^1—X have been noticed (Fig. 3 *e*); the two last associations likewise require a chiasma exchange in the differential arms of Y^1. The association pictured in Fig. 3 e^4 belongs to the same group. Neither in this case it is possible to give an exact estimate of the chiasma frequency in the differential arms, but the proportion seems to be slightly higher than that found previously in the $XXYY$ ♂♂ (WESTERGAARD, 1940).

In the XXY^2Y^2 plants the associations Nos. 8—9—10 occur in about 38 per cent of the cells, the corresponding figures in the $XXXY^2$ plants being 62 per cent. However, the difference is not highly significant ($\chi^2 = 6{,}01$, $P < 0{,}02$) and may be due to mere chance.

The $XXXY^3$ type (Fig. 3 *c*) has not been included in Table 10, since it does not call for special comments; the Y^3 chromosome is univalent in all cells and the three *X* chromosomes are associated in both arms (Fig. 3 c^2).

TABLE 10. (*Cf. Fig. 3*).

	1	2	3	4	5	6	7	8	9	10	Total
$XXXY^1$	3	6	16	1	6	17	1				50
$XXXY^2$		3	12		3	3		6	20	5	52
XXY^1Y^1	2	21	28	4	20			2	3		80
XXY^2Y^2	1	8	14	1	5	3		7	7	5	51

TABLE 11. (*Cf. Fig. 4 and Plate II e—i*).

	11	12	13	14	15	16	Total
XYY^1	36[a]				21		57
XYY^2		6[b]	21[c]	28[d]	1		56
XYY^3	88[f]		2[e]				90
XXY^1		5	11	35	1		52
XXY^2		7	20	26	1	1	55

[a] *X*—*Y* or Y^1—*Y* with univalent Y^1 or *X*: 23 cells; *X*—Y^1 with univalent *Y*: 13 cells. [b] *Y* univalent. [c] *X*—Y^2—*Y* or Y^2—*X*—*Y*. [d] $\langle \frac{X}{Y^2} \rangle$—*Y*. [e] *X*—*Y*—$Y^3$. [f] Y^3 univalent.

The associations in the XYY^1, XYY^2, and XYY^3 males are pictured in Fig. 4 and Plate II, and the statistical results are shown in Table 11. These associations are very important, since they show the homology between the aberrant *Y* chromosomes and both the normal sex chromosomes. These associations prove that

(1) one arm of Y^1 is homologous with the homologous arms of *X* and *Y*, the other arm is not homologous with any arms of *X* or *Y*, since the only trisome observed (No. 15) requires a triple chiasma in the homologous arms of the three chromosomes;

(2) one arm of Y^2 is homologous with the homologous arms of *X*

and Y, the other arm is homologous with the differential arm of X, but not with the differential arm of Y (associations Nos. 12, 13 and 14);

(3) one arm of Y^3 is homologous with the differential arm of Y (the X—Y—Y^3 chain), the other arm is not homologous with any arm of the normal sex chromosomes.

Finally, it should be mentioned that the associations found in the XXY^1 and XXY^2 intersexes (cf. Table 11) are fully consistent with the above conclusions. Hence, the following gametes are to be expected from the plants: 1 XX : 1 XY^1 : 1 X : 1 Y^1 and, by selfing, the following sex chromosome combinations are possible: 1 $XXXX$: 2 XXX : 1 XX : : 2 $XXXY^1$: 4 XXY^1 : 2 XY^1 : 1 XXY^1Y^1 : 2 XY^1Y^1 : 1 Y^1Y^1. (In the mentioned combinations Y^1 may be substituted by Y^2.)

IV. CONCLUSIONS.

The main conclusions from the genetical and cytological analysis of the above material may be summarized as follows (Fig. 5). The presence of Y^1 and Y^2 makes the plants intersexual. The presence of Y^3 leads to male sterility. The long arms of Y^1 and Y^2 correspond to the homologous arm of Y, whereas the long arm of Y^3 corresponds to the differential arm of Y. The short arms of Y^1 and Y^3 are not homologous with any arms of X or Y, whereas the short arm of Y^2 is homologous with the distal part of the differential arm of X. Therefore, it is most reasonable to assume that the Y^1 has arisen from a fragmentation in the differential arm of Y, a long acentric terminal section being lost. In the same way, Y^3 is the result of a fragmentation in the homologous arm of Y. Theoretically, the Y^2 chromosome may also have arisen by a simple fragmentation in the differential arm of Y, the break being nearer to the centromere than the Y^1 break. This, however, involves the fairly improbable conclusion that a section of the differential arm of Y is homologous to the distal part of the differential section of X. If this was true, X—Y bivalents associated by lateral chiasmata should be expected in diploid XY plants, but such associations have never been

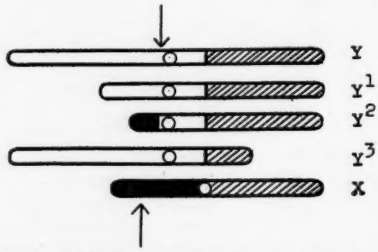


Fig. 5. Diagram showing the constitution of the normal and aberrant sex chromosomes. The homologous arms of X and Y are hatched. The differential arm of Y is white and the differential arm of X is black. The arrows show the two Y^2 breaks.

observed (cf. WESTERGAARD, 1940, pp. 73—75). It seems, therefore, most probable that the short arm of Y^2 actually is identical with the end of the differential section of *X*, e. g., the chromosome is the result of a translocation between the whole differential arm of *Y* and the short distal segment of the differential arm of *X* (cf. Fig. 5). This hypothesis is fully consistent with all observations described above. It would have been strongly supported if the corresponding *X* chromosome with a long differential *Y* segment had been found but, unfortunately, this interesting plant has not been detected.

V. DISCUSSION.

1. THE MECHANISM OF SEX DETERMINATION IN MELANDRIUM.

The recent studies on the sex expression in experimentally produced polyploid *Melandrium* (WARMKE and BLAKESLEE, 1939 a, b; WARMKE and DAVIDSON, 1944; ONO, 1939; WESTERGAARD, 1938, 1940, 1946) have shown that the *Y* chromosome is male determining. KNAPP (1943) has criticized this hypothesis, however, his objections are invalid in the light of the present experience on the intersexuality of the Y^1 and Y^2 plants. In *Melandrium*, sex determination therefore depends upon a balance between the *Y* chromosome and the autosomes + the *X* chromosomes, whereas in *Drosophila* a balance system between the *X* chromosomes and the autosomes is the basis of sex determination (cf. BRIDGES, 1939). The *Melandrium* type has later been found in *Acnida* by MURRAY (1940; quoted from KUHN, 1942; the original paper is not accessible), and probably in *Rumex* subgenus *Acetosella* (A. LÖVE, 1944). In *Lebistes* (cf. WINGE, 1934) and probably in *Lymantria* (cf. WINGE, 1937) the Y chromosome is known to contain sex genes. Although experimental evidence is lacking as in the case of *Rumex*, the *Melandrium* type may also occur in the *Salmonidae* fishes, according to a recent work by SVÄRDSON (1945) and in *Bombyx* (KAWAGUCHI, 1936). The *Drosophila* type has been demonstrated in *Rumex acetosa* (YAMAMOTO, 1938) only, but is generally regarded as the most common type (cf. SUOMALAINEN, 1940; WHITE, 1945), although the above examples do not support this view.

On the basis of the experiences with the Y^1 and Y^2 types it is now possible to approach the problem of the mechanism of sex determination in *Melandrium*. Since the plants are intersexual when the distal part of the differential arm of *Y* is lacking, this section must contain

a gene (or genes) which suppresses the formation of the female organs. However, the plants are still able to exhibit the normal male expression (the anthers seem to be quite normally developed in the intersexual plants), although the distal part of *Y* is lacking; therefore, the rest of the *Y* chromosome must contain a gene (or genes), the presence of which makes the development of the male organs possible. This second gene probably lies in the proximal part of the differential arm of *Y* and, if the translocation hypothesis of the Y^2 chromosome is adopted, it lies between the centromere and the homologous section (cf. Fig. 6). Hence, we are now able to split up the male determining element of *Y* into two separate parts, each affecting the sex expression in a different way, viz. a distal gene [1] which suppresses the female organs and a proximal gene which promotes the male organs. *We call the first gene a female suppressor, since it prevents the development of the female organs without affecting the development of the male organs, whereas the second gene is denoted as a male promoter, since it promotes the formation of the male organs without interfering with the expression of the female organs.*

While the rôle of the *Y* chromosome in the mechanism of sex determination is now fairly well analysed, the problem remains to be solved which is the part played by the *X* chromosomes and the autosomes. Although the final discussion of these points has to be delayed until further experimental evidence is available, the main points of the discussion may already be suggested now. The problems may conveniently be grouped as follows.

(1) Is the *Y* chromosome the only chromosome which contains male determining genes, or are such genes present also in the autosomes?

(2) Are the female determining elements located in the *X* chromosomes, in the autosomes or in both?

(3) Does the female determining element consist of a male suppressor system and a female promoter system, analogous to the male determining system in the *Y* chromosome? And if the question is answered in the affirmative, where are these systems located?

Regarding the first point, it should be emphasized that one of the most important theses of the modern theory of sex determination is the

[1] The presence of an epistatic sex gene in the distal part of *Y* has been suggested previously (WESTERGAARD, 1940) and the low chiasma frequency in the differential arm of *Y* in *XXYY* males was explained on the basis of this hypothesis. Since the XXY^1Y^1 males are also able to form chiasmata in the differential arms (cf. p. 432), this explanation is no longer tenable and the phenomenon is still unexplained.

potency of both sexes to develop the sexual characters of the opposite sex or, in the terms of HARTMANN, »das Gesetz der allgemeinen bisexuellen Potenz» (cf. HARTMANN, 1943, p. 360). The male potencies of the female *Melandrium* are only possible if male genes (male promoters) are present outside the *Y* chromosome. The male potencies of the female plants are not a mere postulate, but are supported by several sources of evidence, viz.

(1) Female plants which are affected by the smut fungus *Ustilago violacea* develop anthers (STRASBURGER et al.; for literature, cf. CORRENS, 1928).

(2) Female plants occasionally possess rudimentary anthers at the base of the flowers (cf. LÖVE and LÖVE, 1945).

(3) Such rudimentary anthers may develop when female flowers are treated with the animal sex hormone testosterone (LÖVE and LÖVE, 1945).

(4) An autosomal gene pulling in the male direction has been described by WINGE (1931).

(5) The occurrence of hypotriploid and hypotetraploid intersexes shows that the absence of certain autosomes may pull the development in female direction (WESTERGAARD, 1940). In this connection, the *Melandrium rubrum* hermaphrodite described by ÅKERLUND (1927) may be mentioned but, in the present author's opinion, it seems more probable that ÅKERLUND's *XX* plant actually contained a fragmented *Y* chromosome or was a translocation hermaphrodite (cf. D. LÖVE, 1942).

Regarding the localization of the female determining element, WARMKE and BLAKESLEE (1939 a, b) and WINGE (1931) hold the view that the sex chromosomes are the only chromosomes which contain sex determining genes of importance (cf. KUHN, 1942), whereas the present author presumes that the female genes lie in the autosomes and in the *X* chromosomes (WESTERGAARD, 1940). Since aneuploid intersexes with different *XY* combinations (for instance, *XXY*—*XXXY* or *XXYY*) occur in the offspring of triploid *Melandrium* (WESTERGAARD, unpublished), the latter explanation seems to be correct, but the discussion on this point should be postponed until this triploid material has been published.

Finally, regarding the possibility of the occurrence of a »promoter-suppressor» system in the female determining element, experimental evidence on this important point is lacking completely, but roentgen experiments with the aim of producing aberrant *X* chromosomes are now in progress. However, it seems not at all improbable that the differential section of *X* contains a male suppressor, hypostatic to the

male promoter gene in *Y*, but epistatic to the male promoting genes in the autosomes. This would explain the discrepancy between the fact that so far no *XX* intersexes have been detected in the offspring of triploid *Melandrium*, although male promoting genes evidently are present in the autosomes. It is in fact surprising that external agents like the smut fungus *Ustilago* are able to neutralize this (postulated) female suppressor which is absolutely epistatic in the unaffected females. An examination of the mode of action of the fungus seems to be greatly needed (cf. ERLENMEYER and GEIGER-HUBER, 1935).

Summarizing, the sex determination in *Melandrium* may depend upon the interaction between the following systems:

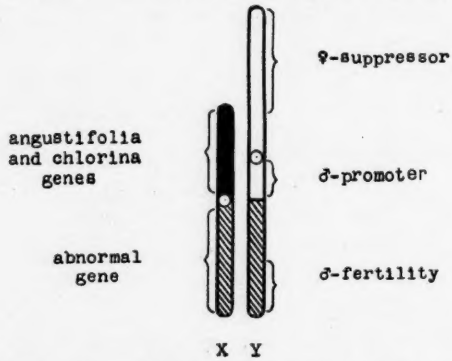


Fig. 6. Diagram showing our present knowledge about the genetical constitution of the sex chromosomes of *Melandrium* based upon the work of WINGE (1931) and the present investigation.

(1) A male promoter in the proximal part of the differential arm of *Y*, epistatic to the (postulated) male suppressor in *X*.

(2) Male promoters in the autosomes, hypostatic to the male suppressor in *X*.

(3) A female suppressor in the distal part of the differential arm of *Y*, partly epistatic to the female promoters, since an accumulation of female promoting genes in the aneuploid intersexes partly may inhibit the effect of the female suppressor.

(4) Female promoters in the autosomes and probably in *X*.

(5) Probably a male suppressor in the differential section of *X*.

The male sterility of the $XXXY^3$ plants shows that the distal part of the homologous arm of *Y* contains a gene (or genes) the presence of which is necessary for the normal development of the anthers. It will always be a matter of definition whether such genes should be called sex genes or not. We may call all those genes sex genes the absence of which or the mutant allelomorphs of which in some way or other influence upon the normal sex expression of the individual, or the term should only be applied to those genes the presence of which in the differential segments of the sex chromosomes is indispensable if the mechanism of sex determination shall work in a normal way. It must always be kept

in mind that sex (maleness or femaleness) is a character which, although normally inherited as a qualitative monofactorial segregating character, has a quantitative, polygenic basis. Therefore, the genetics of sex shares the fate of polygeneous genetics in the whole. Generally, we ignore how many genes are active, we ignore the exact localization of these genes, and we do not know the effect of the individual gene. The more we know about the last point, fhe better we can adequately describe the genes. Before the aberrant *Y* chromosomes in *Melandrium* were known, we would have called the sex gene in the *Y* chromosome a male determining gene (genes); now, it is possible to show that one section has a highly specific effect in suppressing the female organs. The *AGZ* symbols or *MF* symbols used by many German authors veil these problems instead of defining them and, to some extent, the same applies to the index system used by BRIDGES, since sex is determined or influenced by many genes some of which have a cumulative effect, others being more or less epistatic.

Our present knowledge as to the genetics of the sex chromosomes of *Melandrium* is shown in Fig. 6.

2. THE EVOLUTION OF DIOECISM.

The final aim of every analysis of the mechanism of sex determination is a better understanding of the evolution of dioecism. In *Melandrium* dioecism unquestionably has arisen from the bisexual condition found in the related Silenoideae (cf. BLACKBURN, 1929). This problem is one of the most attractive aspects of the investigation now in progress.

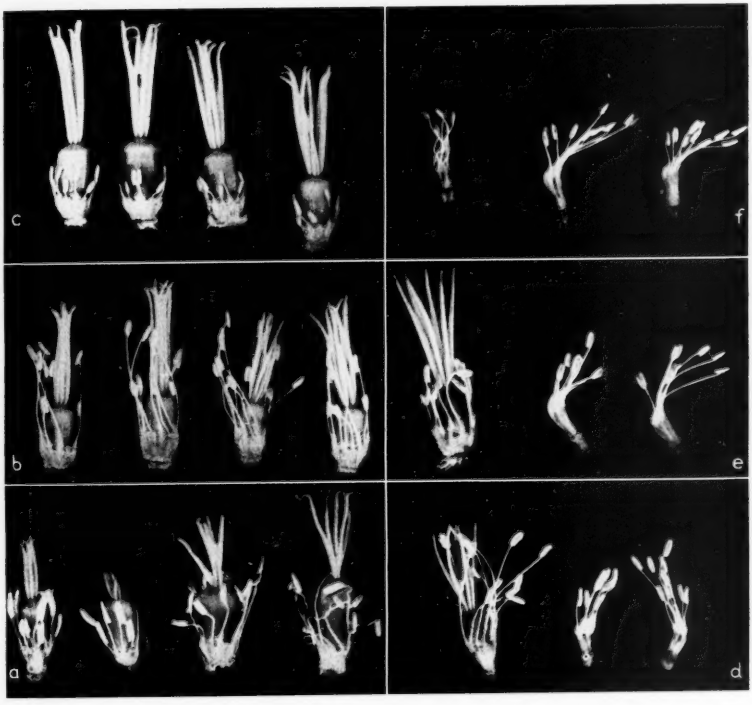
At the present state of the investigation, in dim outline, we imagine that the sex mechanism depends upon the interaction of a system of sex promoting genes distributed over all chromosomes, and sex suppressors —lying first and foremost in the differential arms of the sex chromosomes — the presence of which suppresses the sex promoters of the opposite sex. It is most probable that the sex promoting genes represent the original system, present also in the bisexual condition, whereas the evolution of the sex suppressors is closely linked to the evolution of dioecism. The origin of the epistatic female suppressor (by mutation) has probably been an important step in the genetic evolution of dioecism, and we may imagine that a translocation of the mutant gene (or genes?) to another chromosome has been a further step in the evolution of the heteromorphic sex chromosomes.

The possibility of a stable reversion to hermaphroditism in poly-

ploid *Melandrium* by loss of the female suppressor in *Y* should be mentioned in this connection, the XXY^1Y^1 and XXY^2Y^2 plants already now being fairly constant by selfing. The bisexual condition in *Empetrum hermaphroditum* may have arisen in this way (cf. WESTERGAARD, 1940).

The most evident demonstration of the evolution of unisexual strains from a bisexual organism has been given in maize by JONES (1934) and EMERSON (1932). The experiences gained on maize mutants closely resemble those obtained in *Melandrium*. In the unisexual maize a number of sex genes (sex promoters) exist, distributed over all chromosomes. By a single gene mutation the »silkless» condition is obtained, this gene acts as a female suppressor. »Tassed seeds» is a second mutation, converting the male flowers of the terminal spikes into pistil flowers, e. g., the gene acts as a male suppressor. Plants homozygous for »tassed seeds» and »silkless» are females, the »silkless» gene being hypostatic to the homozygous »tassed seed» condition. Plants homozygous for »silkless», but heterozygous for »tassed seeds», are males.

In *Drosophila*, sex is determined by the balance between the *X* chromosomes pulling in the female direction and the autosomes pulling in the male direction, except for the small IV chromosome in *D. melanogaster* which pulls in the female direction (cf. BRIDGES, 1939). The *X* chromosome contains many cumulative female genes distributed over the whole active region of the chromosome (DOBZHANSKY and SCHULTZ, 1934; PIPKIN, 1940; quoted from DOBZHANSKY and SPASSKY, 1941; the original paper not being accessible). According to the work of these authors, the genes first and foremost act as female promoters, and the technique used does not allow any conclusions as to whether the *X* chromosome also contains suppressor genes. Recent investigations of diploid *Drosophila* intersexes (*D. virilis*, LEBEDEFF, 1939; STONE, 1942; NEWBY, 1942; *D. pseudoobscura*, DOBZHANSKY and SPASSKY, 1941) give some information on the mode of action of some of the autosomal sex genes. The wild type allelomorphs of the ix^m mutation of LEBEDEFF or the Ix^B mutation of STONE and NEWBY seem to act as male suppressors (cf. the discussion by STONE). In the presence of two *X* chromosomes (the normal female condition) the mutations cause an intersexual development of the individuals. In the presence of one *X* chromosome in the males, the mutant genes are ineffective, and this proves that the genes are without influence on the development of the male organs. Hence, at least three different systems exist: female promoters in *X* (and some of the autosomes?), male promoters in the autosomes, and male sup-



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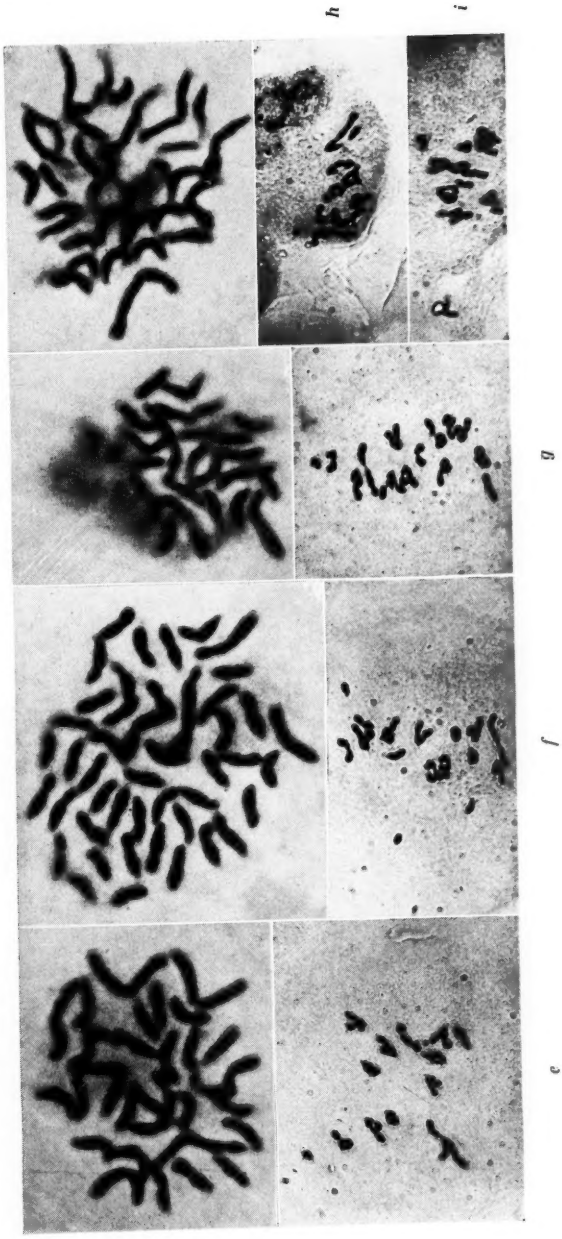

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pressors in the autosomes. The development of the normal females in *D. virilis* depends on a quantitative interaction between the female promoters in *X* and some male suppressors in the autosomes.

SUMMARY.

Three different aberrant *Y* chromosome types have been detected in the offspring of polyploid *Melandrium* (Y^1, Y^2, and Y^3).

Y^1 is the result of a terminal fragmentation in the differential arm of *Y*. The Y^2 chromosome has arisen from a translocation between the differential arms of *X* and *Y*, and Y^3 is the result of a terminal fragmentation in the homologous arm of *Y*.

Plants with Y^1 or Y^2, in which the terminal section of the differential arm of *Y* has been lost, are intersexual, the male and female organs being developed normally. Plants which contain the Y^3 chromosome are male sterile.

It is concluded that the differential arm of *Y* contains two separate male determining elements, a female suppressor in the distal segment and a male promoter in the proximal segment. Evidence is moreover given for the existence of male promoters in the autosomes. Female promoters are found in the autosomes and probably in *X*. The existence of a male suppressor in *X*, hypostatic to the male promoter in *Y*, is suggested.

As is to be expected, by selfing XXY^1Y^1 and XXY^2Y^2 plants, a great excess of intersexes is produced, and the possibilities of producing constant intersexual strains of the above constitutions are pointed out.

The existence of a »promoter-suppressor» system of sex determination in maize and *Drosophila* is discussed.

EXPLANATION OF PLATES I—II.

Plate I.

Flowers from plants with aberrant sex chromosome constitutions, calyx and petals removed. *a*: Four $XXXY^1$ plants with 45, 44, 43 and 43 autosomes respectively. *b*: $42 + XXXY^2$, four flowers from the same plant. *c*: $44 + XXXY^2$, four flowers from the same plant. *d*: XXY^2 ♀, XYY^2 ♂ and XXY ♂. *e*: XXY^1 ♀, XYY^1 ♂ and XXY ♂ *f*: XXY^3 ♂ with degenerative anthers, XYY^3 ♂ and XXY ♂. — About natural size.

Plate II.

a: The normal sex chromosomes in an *XYY* plant, the two large *Y* chromosomes to the right and *X* to the left in the plate. *b*: XXY^2Y^2, the one of the large Y^2 chromosomes at the bottom, the other at four o'clock. *c*: XYY^1, the Y^1 chromosome

at 7 o'clock. *d*: XXY^3, the Y^3 chromosome at the left. *e—f—g*: Meiosis in an XYY^1 male plant, in *e* the large sex chromosome trivalent to the left (cf. Fig. 4 *a*), in *f* the *X—Y* bivalent at the bottom and the univalent Y^1 at about the middle of the plate, in *g* an Y^1—*X* bivalent about the middle and the univalent *Y* at the bottom. Note the centromere constriction on Y^1. *h—i*: Meiosis in an XYY^2 male plant, in *h* the Y^2—*X—Y* chain to the right, in *i* the trivalent sex chromosome association in the middle of the plate; cf. Fig. 4 *b*. Microphotos. *a—d*: From microtome slides stained in FEULGEN gentiane-violet after a LEWITSKY fixation. — Magnification 3000 ×. *e—i*: From aceto-carmine squash, after a CARNOY fixation. — Magnification 1100 ×.

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THE PLANT SPECIES IN RELATION TO POLYPLOIDY AND APOMIXIS

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ACCORDING to the view advanced by BECK (1933) the so-called great interglacial (B) separated Glaciopliocene and Pleistocene. It lasted for more than 200.000 years. During this long undisturbed period the plant species were able to conquer large areas. In HULTÉN's version (1937) most arctic and numerous boreal species arose in the Bering Sea region and radiated from there already at preglacial or at least B-interglacial times. Depending on their different rates of spreading they formed »equiformal progressive areas». Some of them even acquired a continuous circumpolar distribution. (To this hypothesis important objections have been raised by NANNFELDT, 1940, and SAMUELSSON, 1943).

This long period of dispersion broke down with the events of the Riss glaciations, followed after short intervals by another two or three Würm glaciations. The populations became exposed to repeated contractions and expansions. The present status of a species is the result of incidents that took place thousands of years ago.

The first to happen was that the wide distribution areas were cut down into separated units. The biotype material was severely reduced. If the gene material present allowed it, special ecotypes standing harsher conditions arose. Soon, they became annihilated too. South or north of the ice, on nunatakks or in ice-free sheltered places, splinters of the original populations were able to lead a languishing life. In the interglacials these remnants tried to spread and join one another, but then had to undergo the processes of contraction again.

Cross-breeding species are stuffed with lethals of different strength (this is a well-known fact). The isolated communities cut down from the original populations successively homozygotized, vigorous heterozygotes disappeared, inbreeding degeneration ensued, lethal and sublethal homozygotes increased in number. If by any chance however a polyploid arose, the lethals immediately lost most of their destructive effect ($Aa \rightarrow AAaa$); inbreeding would imply no impending danger and, what is more, the cross-breeding populations would change more or

less to self-fertilizing. (This latter effect of polyploidy was stressed especially by LEVAN, 1936, but also pointed out by MÜNTZING in 1930.) When better times approached, the diploid biotypes, if still alive, were not able to spread again, new ecotypes could not form. Instead, the polyploids possessing favourable genes in double dose (for instance, selected genes for cold-hardiness) but lacking the destructive effects of the lethals, rapidly conquered the new areas liberated by the melting of the ice (cf. GAMS, 1933).

Seen in this light, the natural polyploids are not superior to the *original* populations. Of that we know nothing. The diploids get destroyed because their mechanism of heterosis betrays them; in large populations cross-breeding (and its consequences) means an advantage, in isolated remnants it implies destruction. Thus, what should be compared from the viability point of view, is not the polyploid status itself *contra* the diploid, but rather the polyploid biotypes *contra* the invigorated sum of inbred diploids still alive.

Another fundamental question must be touched upon here. If a low-land species, say a boreal type, manages to grow and flower below its temperature optimum, its meiosis subject to repeated cold shocks, unreduced gametes will be formed in great number. (See, for instance, the paper of ROSENBERG, 1946.) These will be continually fed into the population. For this reason many diploids will *automatically* give way to tetraploids and these to hexaploids or octoploids, thus in no way disappearing owing to any fundamental inferiority in viability. Those species, however, that are adapted by special physiological properties to this kind of environment will remain diploid.

Let us regard this problem more closely. Take for instance the *Carices* of *one limited flora region*, the Scandinavian. Some *Carex* species are restricted to alpine and high-alpine localities, others have a northern but not alpine distribution, others must be regarded as low-land plants. The average chromosome numbers of these three groups are $2n = 62$ (11 species), $2n = 57$ (10 species), $2n = 63$ (56 species). Mountain species show no higher average chromosome number than low-land plants. Similar is the case with the Scandinavian *Salices*. Four out of six alpines, five out of eight northern species and six out of ten southern species are diploid: rather a surplus of diploids in the alpine group. The species of *Ranunculus* and *Saxifraga* behave in a similar way.

In some instances the alpine or subalpine species even represent a low-chromosomal status, while the polyploids occur among the southern

representatives. So is the case with the few Scandinavian mountain-plants of *Veronica, Sedum, Cardamine, Viola.*

Further, there is a great number of low-land plants, occurring also in Scandinavia, that have very high chromosome numbers: *Isoetes lacustris* (2n up to 105), *Ophioglossum vulgatum* (2n up to 344), *Sieglingia decumbens* (2n up to 124), *Juncus bufonius* var. *ranarius* (2n up to 120), *Scirpus maritimus* and *caespitosus* (2n up to 110), *Orchis Traunsteineri* (2n up to 122), *Rumex hydrolapathum, aquaticus* and *maximus* (2n up to 200), *Stellaria palustris* (2n up to 130), *Cerastium holosteoides* (2n = 144), *Nymphaea alba* and *candida* (2n up to 160), *Ranunculus Lingua* (2n up to 128), *Trifolium medium* (2n up to 130), *Trientalis europaea* (2n up to 160). Other low-land plants behaving in the same manner are found in the lists of DARLINGTON and JANAKI-AMMAL (1945).

These examples (which could be multiplied) show that the alleged correlation of polyploidy to latitude or altitude implies a highly complex situation. The inferences on polyploid superiority, involving, for instance, improved cold-hardiness effected by the polyploid state itself, must be taken with great caution. Low-land species may be as high-polyploid as any alpines. For a fair comparison every genus and every flora region must be examined separately. The single means by which the value of a polyploid can be determined in a satisfactory way, relative to that of its diploid ancestor, is in yield trials of agricultural plants. (This holds true also of mutations.) The happenings in nature are much too complex to allow definite evaluations of the »fitness». For such an evaluation the whole events of the past (the history of the species) must be unrolled.

Seen in this light, the much discussed conditions within *Biscutella* (MANTON, 1937) will get another explanation.

The section Lyratae is diploid throughout and has a large area of distribution around the Mediterranean. All species are self-fertile.

Laevigatae consist of diploid relicts, tetraploids and hexaploids. The diploids have one alpine off-shoot, very local. Most of them are low-land plants and interglacial remnants (MANTON). All are highly self-sterile. The tetraploids have gained a large area, profoundly alpine and post-glacial in origin.

These results may be interpreted as follows. The original diploid populations of Laevigatae survived in sheltered places, but in few individuals, suffering from homozygotization and inbreeding. Tetraploids, arisen prior to this degeneration, had no lethal effects. In addition.

they obtained a powerful vegetative reproduction (by root-buds) that counterbalanced the still existing self-sterility (?). When new soil successively opened up, they easily overcame the stagnant inbred diploids. The viability of the self-fertile Lyratae, on the other hand, was not damaged by a decreased number of individuals. No degeneration set in. Polyploids — which also here must have arisen now and then — did not imply an essential improvement.

The rough sketch given above was originally drawn up in connection with the European blackberries (GUSTAFSSON, 1943). Most of these, and especially the northern ones, are now apomictic. Comparative studies have shown that the sexual relatives of most apomictic phanerogams are dioecious or monoecious, self-sterile (physiologically dioecious), as proved by genetical experiments, or in any case flower structure suggests cross-pollination. (A parallel state of things holds good also of moss-species reproducing vegetatively.)

Previous to the glaciation (or glaciations) different sexual species of *Rubus* (to exemplify by this genus) inhabited and mixed over all Europe. When the populations thinned out and only solitary clones survived, the self-sterility occurring rendered reproduction of the different species difficult, the interspecific hybrids increased in number and recombinations were facilitated. Apomictic reproduction gave the hybrid derivatives, which were polyploid, an easy victory. In refuges within the glaciated regions and in tracts south and east of the ice-sheet apomictic segregates and polyploids gained the ascendancy. The diploids, especially the northern ones, were largely eradicated, owing to their complex mechanism of fertilization and heterosis, not so much to any original vegetative inferiority. Summarizing we may conclude that cross-breeding diploids generally suffer when occurring in low individual numbers. Apomicts and polyploids are less sensitive under a similar process of population contraction.

These and related problems will be fully discussed in the author's work on »Apomixis in higher plants» (in Lunds Universitets Årsskrift. N. F. Avd. 2. Part I issued in 1946. Parts II—III in preparation).

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HETEROCHROMATY IN CHROMOSOMES DURING THEIR CONTRACTION PHASE

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I. MATERIAL AND METHOD.

DURING the investigations on the cytological effect of different salt solutions on root meristems of *Allium Cepa*, which were made at this laboratory last year (LEVAN, 1945), I noticed that several substances had a definite effect on the stainability of the metaphase and anaphase chromosomes: the stain was kept very stubbornly in the neighbourhood of the centromeres, while the main body of the chromosomes was very weakly stained or quite stainless. The chromosomes of *Allium Cepa* normally show heterochromaty of the spindle region, and these pre-treatments consequently allow a differential staining of the heterochromatin during those stages of the mitosis when the heterochromatin is not distinguishable from the euchromatin with ordinary methods.

In order to ascertain whether this method could be generally used in *Allium*, a number of species was selected for testing from among my *Allium* cultures at Svalöf. The distribution of the heterochromatin within these species was known in its general features from earlier studies. As is well known, the location of the heterochromatin in the chromosomes is studied most favourably at telophase, when the euchromatin begins to lose its colour, although the chromosomes are not yet despiralized and their outlines are still discernible. The staining of the heterochromatin both at telophase and during the resting stage is, however, always whimsical. Rather long experience is therefore required of a certain material in order to attain to a detailed knowledge of the localization of its heterochromatin.

The *Allium* species selected were of two types as regards the quantity and distribution of their heterochromatin: (1) one type poor in heterochromatin with the following species: *Allium cernuum* ($2n = 14$), *Cepa*, *fistulosum*, *nutans* ($2n = 16$), *Schoenoprasum* ($2n = 16$ and 32), *odorum*, *Porrum* ($2n = 32$); and (2) one type rich in heterochromatin with one diploid form of *A. carinatum* ($2n = 16$).

The former type (1) has the main part of its heterochromatin located at the centromeric region. Small heterochromatic portions are present at the chromosome ends, and, in addition, numerous very small grains of heterochromatin are scattered intercalarly in the chromosome arms. In untreated telophases the amount of heterochromatin found depends on the degree of differentiation of the slide: either the heterochromatin is found all over the nuclei, although more densely at the poles (i. e. at the proximal parts of the chromosomes), or, on more advanced differentiation, only at the polar and the equatorial side of the nucleus (at the proximal and distal parts of the chromosomes), or, finally, on still heavier destaining, only at the polar part of the nuclei (at the proximal chromosome parts). There are certain variations between the studied species but their behaviour agrees on the whole. Most *Allium* species behave in this manner, as do also the majority of plant and animal materials.

The second, heterochromatin-rich type (2) is distinctly different from the former type. Its resting nuclei contain great quantities of heterochromatin distributed over a great number of larger and smaller bodies. These often have a long and somewhat irregular shape, indicating that they consist of entire segments of the chromosomes which are heterochromatic and fail to despiralize during the resting stage. On stronger destaining they often turn out not to be homogeneous but to have a grainy consistency. It is harder in this case to discern where the heterochromatin is located on the chromosomes, since the telophase chromosomes are crowded with stained bodies, but it seems to be, above all, the chromosome ends which have received a considerable increase in their charge of heterochromatin as compared with the former type. But large lumps of heterochromatin occur also in the intermediate parts of the chromosome arms. Earlier attempts to achieve differential segments of the chromosomes of *Allium carinatum* by means of cold treatments have failed, as also in the case of other *Allium* species tested.

The method of treatment used in this study was the following. Bulbs were taken from the field in July, 1945, were entirely freed from roots and planted in pots in the greenhouse. When new roots began to grow out, the soil was rinsed away and root-tips were pre-fixed for four hours in 0,005 mol. mercuric nitrate. Thereafter the roots were transferred into NAVASHIN. The reaction in question is attained in such concentrations of mercuric nitrate as lie slightly above the threshold concentration for fixation of the cells, i. e. 0,001—0,01 mol. Since

some basic salt is precipitated at the preparation of the solution, the real concentrations are probably somewhat inferior. The root-tips were cut (mainly longitudinally) and stained in crystal-violet in the ordinary manner.

In addition to the experiments on *Allium* species, the method was tested more cursorily on some other materials, *Secale*, *Hordeum*, *Funkia*, *Lilium*, *Dipcadi*, and *Iris*. Although clear tendencies to differential staining of the heterochromatin were met with in most of the treated plants, definitive demonstration of the same reaction as in *Allium* was obtained only in the case of *Dipcadi serotinum*. A few data on this plant are given after the description of the results in *Allium*. A treatise on the normal chromosome conditions of *Dipcadi* is found in LEVAN, 1944.

II. OBSERVATIONS.

1. ALLIUM.

In longitudinal sections of roots, treated in the above manner, it will be seen that the interior tissues, the plerome and the interior periblem are not fixed all through. The cells of these parts have been disorganized before the fixing action of the mercuric nitrate has been able to work. The exterior layers of the periblem and the dermatogen, on the other hand, exhibit a beautiful and characteristic fixation. The chromosomes of the resting nuclei often show an unusually complete destaining and their heterochromatin stands out very distinctly against an almost colourless background. Within the tissue thus fixed it may be seen that the contraction stages of the chromosomes, from prophase until telophase, assume a specific appearance. The chromosomes are more or less devoid of stain. In certain fixations this stainlessness is accompanied by an almost complete lack of visible structure; the chromosomes look hyaline and somewhat swollen. In other cases, however, the lack of colour may reveal a very delicate structure of the chromosomes. The destaining affects different parts of the chromosomes differently. Those parts which normally lose their colour at telophase are always destained first. Thus, the heterochromatic sections keep their colour.

The staining of metaphase—anaphase after pre-treatment in mercuric nitrate is subject to great variations; the different species studied show about the same degree of variation. Immediately beside quite colourless metaphases and anaphases, strongly stained divisions

may be found in which no interior details can be discerned. In each slide, however, there usually occur 10—25 cells with good contrasts between euchromatin and heterochromatin at metaphase—anaphase. The method may consequently be employed as a reaction of heterochromatin.

A common feature in all diversities of this staining is that the chromosome portions on both sides of the centromeric constriction are always stained. The chromosome ends, too, often show staining. In *Allium carinatum*, thus, great amounts of heterochromatin reach from the ends far into the chromosome arms. A rather characteristic feature is, however, that the centromeric heterochromatin, or, as it will be referred to in this paper, the prochromosomes, are most resistant to destaining. On heavy destaining all colour will disappear from the chromosomes except from just the region of the prochromosomes. This is also the case in *Allium carinatum*, in which there is evidently decidedly more heterochromatin in other parts of the chromosomes than is found in the centromeric region. Thus, it cannot be a question of an equally active dissolution of the stain from the heterochromatin in different regions, since the stain is first exhausted in the chromosome arms, then at the ends and finally within the prochromosomes. In *Allium carinatum*, where the largest supply of heterochromatin is found towards the chromosome ends, absolutely distinct staining is still left within the prochromosomes after every trace of colour has left the chromosome ends.

In Fig. 1 the morphological picture of this type of stainings may be seen. Fig. 1, *a* is a prophase of *Allium cernuum*. The outlines of the chromosomes may be followed clearly enough to enable the proximal and distal parts of each chromosome to be distinguished. As is often the case with cells in rapidly dividing meristems, the chromosomes are »polarized», all centromeres being gathered to one side of the nucleus and all ends towards the other. The centromeres and the ends are more deeply coloured than the rest of the chromosomes. It is clearly seen that the coloured centromeric regions are double, the division into chromatides involves this region. In some cases it may be seen in addition that each prochromosome consists of four parts, being divided both longitudinally and transversally. The transversal split within the prochromosomes is the centromeric constriction.

At metaphase (Fig. 1, *b*—*f*) the picture is rather varying. The prochromosomes, however, are usually stained, while the end portions and other heterochromatic portions show more irregularity. *Allium*

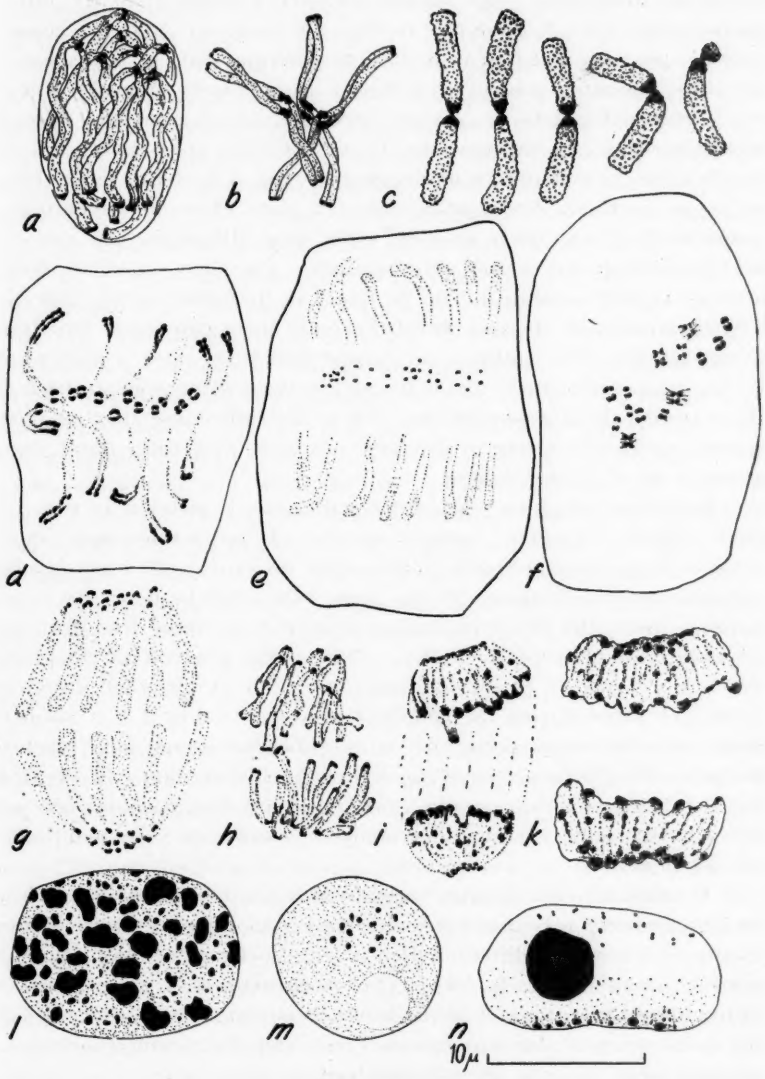

Fig. 1. *a, f, g, m*: *Allium cernuum*, *b, c, k, n*: *A. Cepa*, *d, e, h, i, l*: *A. carinatum*. — *a*: prophase, *b—f*: metaphase, *g—h*: anaphase, *i—k*: telophase, *l—n*: resting nuclei. *c*: after treatment with 0,002 mol. cerious nitrate, all other pictures: after treatment with 0,005 mol. mercuric nitrate. — × 2400.

carinatum may have larger stained terminal portions than the other species (Fig. 1, *d*); it is shown in Fig. 1, *e*, however, that the colour may disappear completely from the chromosome ends of this species, while the prochromosomes nevertheless are distinctly stained. These two pictures illustrate the considerable size variation of the stained prochromosomes in one species. They may vary from about 1 μ in length down to a couple of tenths of a μ. Fig. 1, *b* shows three chromosomes of *Allium Cepa*, taken out of a plate where all prochromosomes and all ends were stained, while very little stain was present in the other parts of the chromosomes. Fig. 1, *c* originates from another type of treatment, viz. 24 hours in 0,002 mol. cerious nitrate. The chromosomes are seen from the pole; their chromatid structure is not visible. The outlines are rugged and they show a somewhat evenly grained structure. On both sides of the centromeric constriction there are darkly stained portions. These are rather undefined at their border towards the rest of the chromosomes, and their distal limit seems to be somewhat hollow.

Beginning anaphase, viewed from the side, is pictured in Fig. 1, *f* from *Allium cernuum*. Several of the 14 prochromosomes show division both longitudinally (chromatid division) and transversally (centromeric constriction). It was quite impossible to detect the chromosome arms, the prochromosomes seemed to be quite free, forming an equatorial plate of their own. During the progress of anaphase the two swarms of prochromosomes are seen at different distances from each other during the anaphase movement. Fig. 1, *g* is an anaphase from the same species, where the prochromosomes have reached the poles. Very little is seen of the rest of the chromosomes. In Fig. 1, *h* from *Allium carinatum*, on the other hand, all chromosome ends are stained, and rather many other stained sections are seen within the chromosomes.

At telophase, when under normal conditions the chromosomes lose their stainability, except in heterochromatic regions, even after mercury treatment a beautiful differentiation is found between euchromatin and heterochromatin (Fig. 1, *i*, *k*). The prochromosomes may be easily distinguished from the rest of the heterochromatin. They are located in the polar part of the telophase nucleus and their centromeric constrictions may often be directly observed.

During the resting stage there is, as pointed out earlier, a striking difference between the species poor in heterochromatin (Fig. 1, *m*, *n*) and *Allium carinatum*, which is rich in heterochromatin (Fig. 1, *l*).

In the latter the nucleus is filled with larger and smaller stained lumps, which condition makes it difficult to distinguish between prochromosomes and what is otherwise located heterochromatin. The amount of stained heterochromatin varies considerably between different nuclei. In such an anaphase as Fig. 1, *i* there is no difficulty in identifying the prochromosomes, but normally there are larger and more irregularly scattered stained portions present. In the heterochromatin-poor species it is often possible to distinguish with certainty between the two kinds of heterochromatin. Sometimes, as in *Allium cernuum* in Fig. 1, *m*, all the heterochromatin has been destained except the prochromosomes. In other cases, as, for instance, in *Allium Cepa* in Fig. 1, *n*, both kinds are present, but they are sharply distinguished from each other both in location and appearance. HEITZ points out (1932) this difference in appearance between the two chromocentra of the *Allium* nucleus: he finds that the distal chromocentra are strongly stained globules, while the proximal ones (the prochromosomes) are more faintly coloured, more diffuse. I have the impression that the present treatment causes the proximal chromocentra to become smaller and to get a sharply defined outline. As is seen from the figure, their centromeric constriction is clearly visible, and they maintain exactly the same appearance as they already have at anaphases such as Fig. 1, *g*.

2. DIPCADI.

In root-tips treated with 0,005 mol. mercuric nitrate for four hours and thereupon fixed in NAVASHIN, exactly the same differential staining was met with as in *Allium*. The occurrence of differentially stained cells in the root-tips agreed with the conditions in *Allium*. Such cells were found in the dermatogen and in the exterior layers of the periblem. In *Dipcadi*, too, the reaction was rather variable, heavily stained metaphases occurring close by metaphases where only the prochromosomes were stained.

Fig. 2, *a*—*c* shows three metaphases in side view. In all of them the prochromosomes are stained while the body of the chromosomes is almost stainless. The coloured portion varies in size considerably. Its size is to some degree proportionate to the general staining of the cell. In the cell which has the smallest prochromosomes (*c*) no traces of the chromosome arms could be detected, while in *a* and *b* the outlines of the whole chromosomes were faintly seen. All prochromosomes

are divided longitudinally, their halves are separated by an up to 1 μ broad stainless cleavage. In *a* and *b* and in some of the prochromosomes of *c* their transversal division can also be seen, although the slides were less clear in this respect than the *Allium* slides. The appearance at anaphase and telophase is seen from Fig. 2, *d* and *e*. These stages are in good agreement with the observations in *Allium*.

In one respect the experiences from *Dipcadi* exceed the findings in *Allium*. Two fixations of root-tips kept for 12 and 36 hours respectively at ± 0 centrigrade showed interesting conditions. These

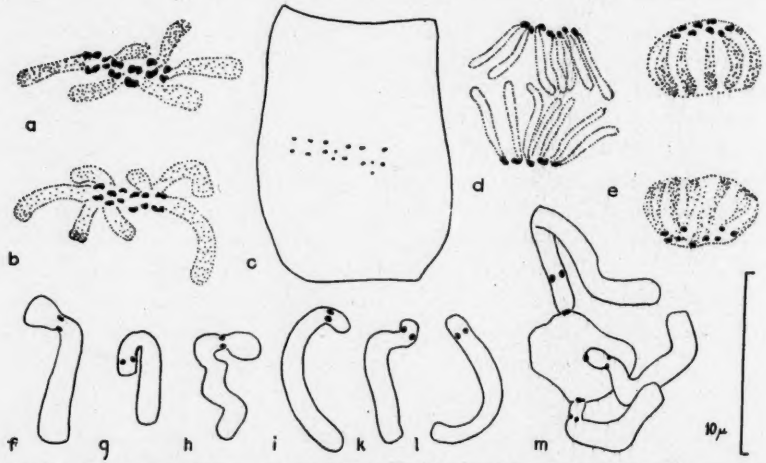

Fig. 2. *Dipcadi serotinum*, *a—c*: metaphase, *d*: anaphase, *e*: telophase, *f—m*: prophase. *a—e*: after treatment with 0,005 mol. mercuric nitrate, *f—m*: after cold treatment. — × 2700.

roots were not pre-treated with mercuric nitrate but solely fixed in NAVASHIN. The cold treatment had effected an almost complete destruction of the spindle, so the mitoses were all of the c-mitotic type. There was also a strong contraction of the chromosomes, as is typical of c-mitosis. The metaphase and anaphase chromosomes were deeply stained, and no differentiations were seen on them except the centromeric constriction. At prophase, on the other hand, the chromosomes, which even at this stage showed stronger contraction than normally, were almost stainless. In each of these very faintly visible chromosomes two strongly stained granules were regularly seen in the centromeric region (Fig. 2, *f—m*). These grains were always lying side by side transversally. Although they were of a very distinct appearance,

I was not able to see with certainty whether they were also double in the longitudinal direction. They are not variable in size in the manner characteristic of the prochromosomal segments, made visible by mercuric nitrate treatment, but they always have about the same size (0,2—0,3 μ). Their form is spherical or somewhat ovate in the longitudinal direction.

As shown earlier (LEVAN, 1944), several or all of the *Dipcadi* chromosomes are connected with the nucleolus by their shorter arm during the resting stage. In the present slides it was seen that four chromosomes at prophase showed a strongly stained narrow band at the point of contact with the nucleolus. Three such chromosomes are pictured in Fig. 2, *m*. All nucleoli show these strongly coloured bands on their surface. These are evidently differentiations in the chromosomes, and probably consist of the satellite region, which is a »nucleolus organizer». Since usually four such stained spots are seen on the nucleoli, it is probable that two chromosome pairs have a special function at the organization of the nucleolus, even if sometimes all chromosomes are seen to be attached to the nucleolus (as, for instance, LEVAN, 1944, Fig. 1, *f*). The two nucleolus-organizing chromosome pairs are probably those which were designated B and C in the cited paper, and which are both provided with satellites.

III. CONSIDERATIONS.

The present investigation has shown that the somatic chromosomes of *Allium* and *Dipcadi* are provided with proximal heterochromatic differentiations. Thus, by various treatments the chromosomes may get their stainability changed so that the euchromatin loses the stain while the heterochromatin remains coloured. These heterochromatic parts, which during the contraction phase of the mitotic cycle cannot be distinguished as a rule from the rest of the chromosome, acquire on this staining a similar appearance to that they normally have during the despiralization phase. A diversity in reaction has been observed between the heterochromatin of the centromeric region and the rest of the heterochromatin: the centromeric region keeps the stain more intensely than the other heterochromatin. The centric regions then assume during metaphase—anaphase the same dumb-bell shape as they normally have during the resting stage. The furrow in this dumb-bell formation is the centromeric constriction as

has been beautifully demonstrated in the prochromosomes of many objects by DOUTRELIGNE (1933).

In order to make a distinction between the heterochromatin of the centric region and that of other parts of the chromosomes, I have above used the term prochromosomes and prochromosomic heterochromatin (OVERTON, 1905; ROSENBERG, 1909). This term appears to be preferable to the somewhat heavier term microchromosomic chromocentra (HEITZ, 1932) and euchromocentra (GREGOIRE, 1932), the latter of which may suggest the presence of euchromatin rather than heterochromatin. The term prochromosome, as used here, thus signifies that part of the heterochromatin which is situated on either side of the centromeric constriction. Since it has been shown that the prochromosomes or in any case parts of them behave differently in staining qualities from ordinary heterochromatin, it may be inferred that this heterochromatin has a special function in connection with the work of the centromeres. It is evidently a part of the centromeric apparatus. Many observations on varying materials which show that the centromeric constriction is surrounded by heterochromatin make this assumption plausible.

At a cursory examination of the literature on the chromosome morphology of different plants and animals rather numerous instances are met with of a deviating behaviour in the staining capacity of the centromeric regions from the rest of the chromosomes. Without aiming at completeness I have in Table 1 collected cases in which the centromeric regions have been found to exhibit differential stainability during the contraction stages of the chromosome cycle.

On examination of these cases it is found that they are mainly of two types: (1) cases where a portion of the chromosome arms of each side of the centromere has been stained, and (2) cases where only a small, longitudinally usually undivided but transversally double body shows colour. In the former case (1) the various authors have often interpreted the stained substance as heterochromatin, in the latter (2) it is often supposed that the centromere itself is made visible (Leitkörperchen, polar granule, granule proximale, kinetochore, spindle spherule). The pictures obtained in group (1) often agree closely with those obtained in the present investigation after mercuric nitrate treatment, while the pictures of *Dipcadi* after cold treatment are definitively of type (2). The question is whether these two types only represent two aspects of the same phenomenon or if there is a real qualitative difference between the chromosome parts stained in (1) and (2). In

TABLE 1. *Cases of differential staining of the centric region.*

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Case No.	Author	Year	Material	Stage		Fixation						Staining					Chromosome part stained
				Mitosis	Meiosis	Pre-treatment	Osmium	Platina	Mercury	No heavy metals	Aceto-carmine	Haematoxylin	Brazilin	Gentian violet	Chondriosomic stain	Heavy destaining	
1	METZNER	1894	*Salamandra*	+	+		+					+			+		Leitkörperchen
2	PINNEY	1908	*Phrynotettix*	+	+							+					polar granule
3	VEJDOVSKY	1912	*Declicus*	+					+				+				Verdickung der Spindelfasern
4	WENRICH	1916	*Phrynotettix*	+	+		+					+		+			polar granule
5	JANSSENS	1924	*Stethophyma, Chorthippus*	+	+		+	+		+							granule proximale
6	NAVASHIN	1927	*Gallonia*	+				+				+				+	Leitkörperchen
7	MINOUCHI	»	*Mus*		+		+					+					polar granule
8	NAKAMURA, K.	1928	*Natrix*		+		+					+				+	polar granule
9	BELAR	1929	*Chorthippus*	+	+		+					+			+		Spindelinsertionsstelle
10	NAKAMURA, T.	»	*Cycas*		+		+					+					polar granule
11	TRANKOWSKY	1930	*Bellevalia, Najas, Crepis*	+						+		+				+	Leitkörperchen
12	NAKAMURA, K.	1931	*Eumeces*	+	+		+					+				+	polar granule
13	WILSON	»	*Opisthacanthus*		+		+								+		polar granule
14	HEITZ	1932	*Allium*	+			+								+		heterochromatin
15	HEITZ	1933	*Drosophila*	+							+						heterochromatin
16	MAKINO	1934	*Pungitius*	+	+		+					+				+	polar granule
17	SCHRADER	1936	*Amphiuma, Mus*		+		+					+			+	+	kinetochore, spindle spherule
18	MATTHEY	»	*Mus*	+			+					+				+	accrochement
19	DARLINGTON	1937	*Tradescantia*		+	+	+							+			centromere
20	KOSTOFF	1938	*Triticum, Crepis*	+			+	+		+		+		+		+	heterochromatin
21	HAMEL	»	*Gronovia, Mengelia*	+					+			+					euchromocentre
22	COREY	»	various *Acrididae*	+	+					+		+					polar granule
23	MATTHEY	»	*Epimus, Arvicola, Apodemus*		+		+					+				+	centromere
24	KOSTOFF	1939	*Nicotiana*		+												heterochromatin
25	SCHRADER	»	*Amphiuma, Plethodon, Tradescantia*		+		+					+			+	+	kinetochore, spindle spherule
26	BENOIT and KEHL	»	*Mus, Sus, Felix*	+	+		+					+			+		centromere
27	IWATA	1940	*Trillium, Lilium*		+	+	+					+					attachment chromomere
28	CALLAN	»	*Anilocra*	+	+		+							+			centric region
29	MAKINO	»	*Drosophila*	+							+						heterochromatin
30	TOGBY	1943	*Crepis*	+			+							+		+	heterochromatin
31	GEITLER	1944	*Paris, Psophus, Gomphocerus*	+	+	+					+						heterochromatin
32	LEVAN	present paper	*Allium, Dipcadi*	+					+	+				+			heterochromatin
			Dipcadi	+		+				+				+			centromere

the former case the variation in appearance between (1) and (2) might be due to uncertainty in observation of such small details, and to the fact that smaller or larger parts of the centromeric region may be stained on different occasions. In the second alternative there might be a real difference between the parts stained: in (1) the stained portion may be the heterochromatin on both sides of the centromere, while in (2) the centromeres, *sensu strictu*, may be stained.

In some of the cases found in Table 1 rather large portions of the chromosomes are stained even among those cases in which the writers tend to consider the stained sections as involving only the centromeres. Among these cases belong NAVASHIN's picture of *Galtonia* (1927, Fig. 3). Certain of MATTHEY's pictures (1938), e. g., of *Apodemus sylvaticus* and *agraricus* also show rather large stained portions. In Plate III, Figs. 1—3 of that paper, representing metaphase I of *Epimys norwegicus*, there is a continuous series from very small single granules in Fig. 1 to decidedly larger, often longitudinally split portions in Fig. 3. Such pictures as these, which also occurred in my material of *Allium*, support the view that only quantitative differences may exist between the small spindle spherules and the larger two-armed prochromosomes. IWATA's paper (1940) also gives instances of longitudinally split portions, for instance, the anaphase chromosome of *Trillium* in Fig. 13, which has the same general type as the prochromosomes of the *Allium* anaphases in the present paper.

CALLAN (1940), who pictures similar formations in *Anilocra*, puts this problem in the following way: »What relation the centromeres, as seen fixed and stained, bear to their size and shape in life is a question of interpretation. It may be argued that what can be stained is really the region of the chromosome near to and including the centromere, not the latter alone. However, its discreteness in stainability and its similar size and shape in all the chromosomes suggest that the artefact is at least an artefact of the centromere itself, and it is of interest to find that in *Anilocra* there is this clear-cut evidence of a distinction between the centric and non-centric regions of the chromosome« (l. c., pp. 334—335). As shown above, the differential segment of *Allium* and *Dipcadi* occurring after mercuric nitrate treatment usually showed the same size and appearance in all chromosomes of the same cell. Their size varies, however, within wide limits between different cells. This latter condition is not valid in the centromeric bodies stained in *Dipcadi* after cold treatment, as well as in most of the cases gathered here in the above group (2), i. e. stainings of the

centromeres. These bodies are usually of about uniform size in the same material, and this condition may be considered as a support for the opinion that qualitatively different chromosomic sections are stained in groups (1) and (2).

In other material collected in Table 1 we find it clearly stated that the stained region is the chromosomic section on both sides of the centromeric constriction. This is true of HEITZ (1932). His heterochromatic type B 2 (l. c., p. 628) »mikrochromosomische Chromocentren» seem to correspond most closely to the prochromosomic heterochromatin, even if his type A »telophasogenes Heterochromatin» often involves the centric region. Anyhow, these two types do not exclude each other. Also the heterochromatin stained in somatic *Drosophila* chromosomes (HEITZ, 1933; MAKINO, 1940) evidently often corresponds to the prochromosomic heterochromatin. As is well known, the salivary-gland chromosomes show heterochromatin at their centric regions.

HEITZ gives a picture of anaphase chromosomes in *Allium Cepa* (1932, Abb. 10, especially 10 *e*), where certainly the same section of the *Allium* chromosome has been stained as after the use of my method. After the technique used by HEITZ (fixing in FLEMMING—BENDA without acetic acid, staining in acid fuchsine) the differential staining seems to be rather irregular: »Ob auch in anaphasischen Chromosomen die Chromocentren schon zu erkennen sind, konnte ich nicht mit Sicherheit entscheiden. Wohl sieht man manchmal besonders stark gefärbte Chromosomenenden, sowohl distale (Abb. 10, *a*, *e*) als auch proximale neben den mehr oder weniger stark gefärbten 'Chromomeren'. Aber die Bilder treten nicht so gesetzmässig auf, wie man das erwarten sollte» (l. c., p. 595).

KOSTOFF (1938) finds that large portions at the centromeres, as also at the chromosome ends, in *Crepis capillaris* and also, although less regularly, in *Triticum monococcum* have a tendency to retain the stain more stubbornly than the rest of the chromosomes. On heavier destaining the chromosomes behaved similarly as in *Allium*: firstly the colour left the intercalary parts of the chromosome arms, thereafter the distal parts and lastly the proximal. »This differentiation seems to be a real one depending on the biochemical nature of the chromosomes, some regions of the latter being able to absorb and retain dyes for longer time, while others getting somewhat easily distained» (l. c., p. 219). In a later work (1939) on amphidiploids of *Nicotiana glauca* × *Langsdorffii* KOSTOFF develops the thought further that

heterochromatin keeps the stain more intensely than euchromatin. The chromosomes of *Nicotiana glauca* are larger than those of *N. Langsdorffii* and are stained deeper than the latter ones when observed in the pure species. This condition is interpreted by the writer as an indication that they contain a greater amount of heterochromatin than the *Langsdorffii* chromosomes. In F_1 some univalents show a weaker intensity of colour and in the amphidiploids some bivalents are normally stained, while certain bivalents become very faint and transparent. In these faint bivalents, which possibly consist of *Langsdorffii* chromosomes, the spindle regions are often of a dark colour. If the weakness of the staining in these bivalents is caused by a smaller content of heterochromatin, it seems also here that a difference in staining reaction is found between the prochromosomic heterochromatin and the ordinary heterochromatin.

In another *Crepis* species, viz. *Cr. neglecta*, TOBGY (1943) obtained differential staining of the prochromosomic regions of somatic chromosomes. Although evidently very many heterochromatic segments were present in the resting nuclei of this species, only the regions on both sides of the centromeres remained coloured after »careful» destaining, just as was the case in *Allium carinatum*. In one of the small chromosome pairs (C_N) the whole chromosome body was heterochromatic.

GEITLER (1944) observed within two genera of grasshoppers that the centromeric region is heterochromatic. He states that this proximal heterochromatin consists »aus einem besonders dichten, stark färbbaren und kaum veränderlichen Heterochromatin; das Heterochromatin des langen Armes des X-Chromosoms erscheint dagegen dem Euchromatin angenähert» (l. c., p. 533). GEITLER's picture (Abb. 1 *h*, p. 532) of somatic metaphase chromosomes of *Gomphocerus* very much resembles the present *Allium* material. In the same paper GEITLER gives *en passant* the information that the chromosomes of the first pollen mitosis in *Paris* show similar proximal, extremely heterochromatic segments, »die auf dem ersten Blick hin mit Centromeren verwechselt werden können» (l. c., p. 536). In this case there can be no doubt that it is a question of identical differentiations as those found in *Allium*.

Thus, it may be stated that rather many of the cases listed in Table 1 are quite evidently of the same nature as the differential stainings obtained in *Allium* and *Dipcadi* after treatment with mercuric nitrate. On the other hand, some of them must be said to differ

definitely from this type, for instance, the stainings of SCHRADER and WILSON and also my stainings of cold-treated *Dipcadi*. The question must for the present be left unanswered whether these two types are distinguished qualitatively or if they differ only by the size of the segment retaining the colour. Further attempts to stain the centromere in contrast to the proximal heterochromatin may elucidate this point.

That the centromeric apparatus is a compound structure is rather generally assumed. NEBEL (1939) conceives the centromere as built up of three main parts: (1) chromatic kinetic bodies, which may more or less correspond to SCHRADER's spindle spherule and DARLINGTON's centrogenes (1939), (2) a central achromatic body (SCHRADER's commissural cup, DARLINGTON's fibrous coat), and (3) connecting chromomeres, which on both sides connect the centromeric organ with the chromatids. After mercuric nitrate treatment mainly part (3) should be stained. After chondriosomic stainings it is possible that part (1) will show a specific staining. KOSLOFF (1937) has shown by chondriosome fixing of *Allium* that an achromatic section connects the two chromosome arms with each other. This section could be stained by eosine and erythrosine but was devoid of haematoxylin colour if the destaining was continued to a suitable degree. As a rule KOSLOFF did not observe any stained »kinetic body» within this achromatic section, but in one species of *Fritillaria* he succeeded in observing simultaneously the achromatic commissural region and two stained granules within this region lying beside each other in a transversal direction. These evidently correspond to NEBEL's chromatic kinetic bodies.

From Table 1 it may be seen that proximal differentiations in the form of darkly stained segments or bodies have been encountered both at mitosis and meiosis of greatly varying materials. Among plants we note representatives of Gymnosperms (*Cycas*), Monocotyledons (*Najas*, *Tradescantia*, *Bellevalia*, *Dipcadi*, *Galtonia*, *Allium*, *Fritillaria*, *Lilium*, *Paris*, *Trillium*, *Triticum*), and Dicotyledons (*Gronovia*, *Mengelia*, *Nicotiana*, *Crepis*). Among animals similar stainings are met with in Crustacea (*Anilocra*), Insecta (*Drosophila*, *Chorthippus*, *Stethophyma*, *Psophus*, *Gomphocerus*, *Phrynotettix*, *Decticus*, various Acrididae), Arachnoidea (*Opisthacanthus*), Pisces (*Pungitius*), Amphibia (*Amphiuma*, *Plethodon*, *Salamandra*), Reptilia (*Natrix*, *Eumeces*), and Mammalia (*Mus*, *Epimys*, *Arvicola*, *Apodemus*, *Sus*, *Felix*). Even if Liliaceous plants and grasshoppers dominate this collection by number, it must be agreed that the reaction in question is scattered rather at random over the plant and animal kingdoms.

Usually the reaction seems to have been obtained unintentionally as a chance occurrence at the ordinary production of slides. Only in five cases are any definite pre-treatments indicated as favouring the reaction (drying, cold, treatment with distilled water, ammonia alcohol, nitric or hydrochloric acid). GEITLER (1944) thus mentions that pre-treatment with distilled water is a necessary condition in his material for obtaining the differential staining. In my *Dipcadi* case the reaction occurred in two fixations from cold treatments, while I have never come across it in untreated or otherwise treated material.

In most of the cases in Table 1, fixatives containing osmic acid have been used (FLEMMING, BENDA, CHAMPY, MINOUCHI, LA COUR, SMITH). JANSSENS, NAVASHIN and KOSTOFF used platinum chloride formalin with or without acetic acid (HERMANN's fluid). JANSSENS stresses repeatedly that the differential staining of the »granules proximaux» has best success if the fixation is heavy: »Dans un de nos objects qui était resté dans le fixateur HERMANN pendant plusieurs mois, et qui se colorait très difficilement, nons avons même pu observer les *granules terminaux* dans des chromosomes à insertion subterminale, Fig. 255, *d*, ou à insertion moyenne, Fig. 255, *a*, *b*, *c*.» (l. c., p. 241). In the legend to Pl. V, Figs. 112—122, in which these heterochromatic granules are especially plain during meiotic prophase, the author writes: »La fixation a été extrêmement violente, ce qui a permis de colorer les granules proximaux et les axes chromosomiaux à l'exclusion dc tout autre détail» (l. c., p. 345). It may be pointed out in this connection that METZNER obtained his excellent differential stainings after fixing the material in not less than 6 % osmic acid dissolved in 0,6 % sodium chloride.

VEJDOVSKY and HAMEL used fixatives with sublimate (chromic acid-sublimate, HELLY). Since pre-treatment with mercuric nitrate has turned out to be especially useful for obtaining a clear-cut differential staining, it seems possible that fixatives with heavy metal salts favour the reaction in question. However, LEWITSKY's and NAVASHIN's fixatives as well as acetocarmine, with or without prefixing in alcohol-glacial acetic acid, have also given differentiations. Smears of beet-roots made at this laboratory have been sometimes found to give rather clear differential stainings in acetic orcein.

It may be mentioned, in passing, that differential stainings of the heterochromatin were obtained after the following treatments. In some cases only tendencies to this reaction were seen, and in no case was the reaction as clear-cut as after mercuric nitrate.

Substance	Molar concentration
$BeSO_4$	0,2—0,005
$Ti_2(SO_4)_3$	saturated solution
$Co(NO_3)_2$	0,01—0,0005
$Y_2(SO_4)_3$	0,02—0,001
$SbCl_3$	0,02—0,0005
$La(NO_3)_3$	0,001—0,0005
$Ce(NO_3)_3$	0,01—0,001
$Nd(NO_3)_3$	0,0005
$AuCl_3$	0,002—0,0005

These values show a tendency to sinking threshold together with increasing atomic weight of the metals involved. This correlation was also indicated by the c-mitotic thresholds of inorganic salt solutions (LEVAN, 1945).

Of the staining methods of Table 1 HEIDENHAIN's haematoxylin has been used in most cases. Other nucleic stains used are brazilin, gentian violet and crystal violet. In many of the papers it has been pointed out that a strong destaining is a condition for obtaining the reaction. With special chondriosomic stains (BENDA, ALTMANN, KULL) differential stainings of the centromeres have been obtained, in some of which the centromeres are qualitatively different from the surrounding chromosome section (METZNER, SCHRADER, WILSON). On the other hand, by using ALTMANN's staining HEITZ obtained a typical prochromosome staining in *Allium* (HEITZ, 1932, Abb. 10 *e*), i. e. a staining of the heterochromatic portions on both sides of the centromeric constriction. CALLAN has tested FEULGEN stain in slides where differentiation on earlier gentian violet staining was visible. He found that the whole chromosome became equally stained by FEULGEN. The same observation was made in my material. Thus, the lack of colour within large sections of the chromosomes is evidently not due to lack of nucleic acid in these sections.

This short survey of the literature on this subject gives as a result the interesting fact that in many cases the centric region both in plants and animals shows a differential reaction from the rest of the chromosomes even during the contraction stages of the chromosome cycle. To these instances might be added a great many cases of an otherwise deviating behaviour of the centric region, which makes it probable that it contains heterochromatin. The conclusion may be drawn that heterochromatin is an important component of the structure of the centro-

meric apparatus. In view of the special function of the centromere, the same all through the world of organisms, it would be by no means surprising if a specialized type of heterochromatin, somewhat differing in stainability from the rest of the heterochromatin, should participate in the building up of the centromere. Many of the forms listed in Table 1 probably have in conformity to *Allium* heterochromatin at the chromosome ends and in other regions of the chromosomes, although in the great majority of cases it is only the proximal heterochromatin which has been observed during metaphase and anaphase, i. e. the whole or part of the prochromosomic heterochromatin. It may be concluded that this heterochromatin differs from ordinary heterochromatin by having a different chemical structure, or the chromosome may have a deviating structure within the prochromosomic region.

SUMMARY.

After a prefixing for four hours in 0,005 mol. mercuric nitrate followed by fixing in NAVASHIN and ordinary staining in crystal violet the chromosomes of several *Allium* species and of *Dipcadi serotinum* will show a differential staining of the heterochromatin also during the contraction stages of the chromosome cycle. The heterochromatin of the centric region (i. e. the prochromosomic heterochromatin) retains the stain longer than the rest of the heterochromatin, even in species such as *Allium carinatum*, where great amounts of heterochromatin are present in other regions of the chromosomes. The conclusion is drawn that the prochromosomic heterochromatin is of a special kind, necessary for the function of the centromeres. Some earlier cases of differential stainings of the centric region during the contraction stages of the chromosomes are discussed in relation to the present results.

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CHROMOSOME NUMBERS OF TWIN PLANTS OF QUERCUS ROBUR AND FAGUS SILVATICA

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IN 1934 NAMIKAWA and KAWAKAMI made the then much discussed discovery that at times twin plants of *Triticum vulgare* possess different chromosome numbers. The commonest case is that of one of the twin plants having the chromosome number typical of the species and the other having the »triploid». A rarer occurrence is that one of the twins has the »tetraploid» or »haploid» chromosome number. This observation was confirmed by MÜNTZING in 1937 and 1938, who reported twin plants with deviating chromosome numbers in 12 species, belonging to 11 genera. With the exception of one case in *Solanum tuberosum* (LAMM, 1938) the whole of this material consisted of members of the family Gramineae. Twin plants with deviating chromosome numbers in other plants than Gramineae have been further reported by, for instance, BERGSTRÖM (1938) in apple. The caryopsis in the Gramineae is built up of a single carpellary leaf with a single ovule. In *Solanum* and *Pyrus* no polyembryony occurs normally. The occurrence of twins in these genera is thus an abnormality in itself.

In other groups of plants with as a rule one-seeded fruit the conditions are otherwise, there being several ovules in the ovary but usually only one develops into ripe seed. Such a case is to be found, for instance, in the Amentifer family Fagaceae, where we have ovaries consisting of 3—6 carpophylls with two ovules on each carpel, in spite of which the ovary develops normally into a single-seeded nut. Still, fruits with more than one kernel — »philippinas» — are not uncommon and are quite simply interpreted as being due to the ability of more than one of the original ovules to develop further. It may therefore *a priori* be expected that twin plants from such fruits will not exhibit chromosomal deviations, although it cannot be considered as ruled out that here, too, twins may occur which have developed abnormally and have abnormal chromosome numbers.

In order to investigate this matter chromosome number determinations have been made at the Forest Tree Breeding Institute on a rather large number of twin plants of oak (*Quercus robur*) and beech (*Fagus silvatica*). Both *Quercus* and *Fagus* have six ovules in each ovary and thus possess potentialities for giving »sextuplets». Twin seedlings are common. In any case they have not so infrequently been found in *Quercus*, whereas more than two seedlings in one and the same nut is a very uncommon occurrence. There are great individual differences in the frequency of twins. Solitary trees may possess a very high twin frequency. For instance, an oak included in the present material, denoted as 44—13 and characterized by its extremely large acorns (1000 acorns = 9,20 kg., normally about 3,5 kg.), gave in an experiment arranged for another purpose 125 plants per 100 acorns against an average of 83,9 plants per 100 acorns for the other four trees included in the experiment. For determination of chromosome numbers 76 twin pairs, 5 triplets and 1 quadruplet, or altogether 171 plants from this oak, were taken. On account of technical failures the chromosome numbers for 17 plants could not be determined. Of the 154 $2n$-determined plants, 153 had the normal diploid number $2n = 24$ and one the triploid, $2n = 36$.

Further, for the chromosome-number determination of about 75.000 acorns from some hundred different oaks, 314 twin pairs and 31 triplets, making 721 plants, were taken. Of these, the chromosome numbers were determined for 577 plants, while 15 seedlings died before being fixed and in 119 cases the preparation was a failure. For 575 of these plants the $2n$ number was determined as the normal diploid, $2n = 24$, and for 2 plants $2n = 36$ was obtained. Of altogether 729 $2n$-determined twin-quadruplet plants of oak, 726 were thus diploid and 3 triploid, which gives a triploid frequency of 0,41 %.

Twin seedlings are far rarer in beech than in oak. Among about 100.000 beechnuts only 66 were found with twin ovules, and in no case were more than two seedlings found in one nut. Of these 66 twin pairs, the chromosome numbers for both the plants were determined in 38 and for one of the plants in 26; 10 plants have died, and in 20 fixations the chromosome numbers could not be determined. All the 102 determinations gave the normal diploid number $2n = 24$.

It is thus evident that the twin frequency is high in oak and beech, being in any case in oak close upon 20 % in solitary trees, in beech averaging about 6,6 per thousand. For different cereals MÜNTZING (1937) gives 1,44—0,07 ‰. This difference is undoubtedly fully ex-

plained by the fact that *Quercus* and *Fagus* have six ovules and the Gramineae only one. The frequency of twins with deviating chromosome numbers in *Quercus* and *Fagus* is however low, in this material being 0,41 % in *Quercus* and 0 in *Fagus* of 102 plants. For a collected material of 2.201 twin plants from 16 different species with typical uniovular seeds MÜNTZING (1938) finds deviating chromosome numbers in 4,32 % of the twin plants. From this it is clearly seen that the frequency of twin plants with deviating chromosome numbers in plants with several ovules per (apparent) seed is essentially lower than in plants with typical uniovular seeds. Still, it is evident that plants of the former category may also give twin plants with deviating numbers. However, it has not been shown here that these triploids in *Quercus* really have any connexion with the twin ovules. Maybe the frequency of triploids in any oak material whatever is about 0,41 %, and that the triploids originate from unreduced but otherwise normal gametes. To test this hypothesis it would be necessary to determine the chromosome numbers in a large number of oak plants from uni-seedling acorns, as the triploid frequency is so low in the twin plants. But such an investigation would meet with practical difficulties.

Purely deductively, however, one may arrive at the conclusion that a triploid frequency of about 0,4 % can hardly be characteristic of oak in general. If in that case the triploids are not selectively inferior to the diploids, which does not seem to be the case, this percentage of adult oaks in natural stands would be triploid. These trees would scarcely have escaped notice, the oak being in Sweden the object of rather intense observation. It would therefore seem most probable that the triploid oak twins also originate from abnormally developed embryo-sacs, in agreement with MÜNTZING's assumption (1937) for other cases. In any case triploid twins would seem to constitute a source of chromosomal differentiation in *Quercus*. However, the approximately 40 oak species with known chromosome numbers are all diploid, and no deviations seem to have been previously encountered within the species.

From a breeding point of view it is always of interest to raise polyploid forms from cultivated plants. In colchicine treatment we now have such an effective means to this end that the earlier methods for the production of polyploids have no longer the same interest. In the case of the lignoses, however, the position is somewhat different. Tetraploid ligneous plants produced by colchicine treatment have admittedly been reported, e. g. by JENSEN and LEVAN (1941), but so far this has

reference only to C_0-products, which must at bottom be regarded as mixoploid (JOHNSSON and EKLUNDH, 1940). STRAUB (1941) considers that colchicine treatment has small prospects for the lignoses. This view rests on the fact that in the lignoses a long time and innumerable cell generations intervene between treatment and flowering. The original mixoploid C_0-product loses its polyploid tissue on account of the latter's assumed lower speed of cell-division. This applies to seed as well as twig-tip treatment. After seed treatment it must necessarily take a number of years before flowering starts. Twig-tip treatment is still less reliable. Profuse flowering occurs only at intervals of several years in forest trees, and it is therefore always difficult to treat just those branch-tips which will flower the following year. Moreover, the treatment in itself may have an inhibitory effect on the flowering of the branch. In any case its vegetative development is arrested, which is liable to lead to a stifling of the treated branch by those surrounding it. STRAUB is therefore right to some extent.

Extensive colchicine material of various lignoses cultivated at this Institute, a material that is now up to eight years old, suggests however that here, too, tetraploid and triploid plants may be expected in the C_1. However, so far as forest trees are concerned, the older, polyploidy-inducing methods are still of value. This applies especially to large-seeded species such as the oak and beech, the apical buds of which are also very large and multicellular, and therefore the C_0-product cannot be expected to become particularly homogeneous in respect of polyploid tissue.

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BEHAVIOUR ON THE SPINDLE OF THE ACTIVELY MOBILE CHROMOSOME ENDS OF RYE

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INTRODUCTION.

AS is well known, there is in normal cases in a chromosome only a very small region or particle which has the property of active mobility on the spindle; it is called the *centromere*. This particle is responsible for the movement of the chromosomes to the equator of the spindle during metakinesis (prometaphase) and to the poles during anaphase. The rest of the chromosome is passively dragged along by the centromere.

In some strains of inbred rye, however, it was found by KATTERMANN (1939) and PRAKKEN and MÜNTZING (1942) that besides its normal submedian centromere a certain chromosome also had another particle that showed active mobility on the spindle, viz. one chromosome end. As was pointed out by PRAKKEN and MÜNTZING, this end differed from a centromere in important properties; so the active component cannot be considered as simply a translocated centromere (as initially suggested by KATTERMANN). From these studies it also appeared that the phenomenon resulted from the interaction of two different causes, viz. a special *structural* differentiation in this chromosome end, and a special, genotypically and environmentally controlled, *physiological condition* in the cell. The peculiarity was manifested only during the two divisions of meiosis. The arms of the chromosome in question are different in length. The characteristic effect appeared in the end of the short arm. This chromosome with the mobile end was called the T-chromosome in accordance with the terminology of KATTERMANN (1939).

Four strains differing in intensity of the T-effect belonged to the above-mentioned type with this effect manifested only in one of the seven chromosome pairs. In one special strain, however, there was a general tendency of all the chromosomes to behave in this way; the effect could be seen in a number of chromosome pairs varying (between

cells) from 0 to 6. Here, too, it seemed to be the ends of the short arms which pointed to the poles. This may indicate that the ability to behave in this way is a general property of the short arm chromosome ends of rye, which is manifested under certain physiological conditions, and which may be more easily manifested in ends that have been subjected to suitable structural changes. According to LEVAN (1942), these ends of the short chromosome arms are heterochromatic.

There has been a great deal of discussion concerning the nature of the interaction between centromeres and spindle poles, whether it should best be described as a repulsion or as an attraction. In the case of the *T-ends* of rye PRAKKEN and MÜNTZING concluded that they were *attracted* towards the poles. This is undoubtedly very clearly evidenced by their observations. The end is pointed and directed towards a spindle pole, and the short chromosome arm is clearly seen to be subjected to a strong tension. Furthermore, the bivalents are often strongly displaced from the metaphase plate [1] towards that pole towards which the ends are directed. PRAKKEN and MÜNTZING point out that these observations need not mean that the forces working at centromeres are of the same type. These authors, however, do not concentrate much of their attention on this aspect of the problem. The behaviour of T-ends and centromeres on the spindle is no doubt worthy of a more detailed comparative investigation. This is the purpose of the present paper.

Recent observations on the equilibrium position of trivalents during metaphase of the first division of meiosis by ÖSTERGREN (1945) and BÖÖK (1945) indicate that the forces working on centromeres are best described as attractions to the spindle poles. According to ÖSTERGREN (1945 a and b), these attractions are of the peculiar kind that they become stronger the greater the distance is between centromere and pole. And, furthermore, the anaphase centromere is attracted only by that pole towards which it is turned; its orientation thus being of great importance. The metaphase centromere in mitosis is considered as composed essentially of two anaphase centromeres turned in opposite directions.

[1] It should be noted that when we use the term »*displaced from the metaphase plate*» in this paper it is always only to designate that the chromosome configuration has a position deviating from that of the normal bivalents in lying outside their metaphase plate. We do not intend to suggest by our term that the chromosomes in question have been in the metaphase plate and later been removed from it. Most probably such configurations have never been in the plate but have gone direct to their abnormal metaphase position during the prometaphase movements.

Considering that there is thus evidence that the centromeres also, just like the T-ends of rye, are *attracted* to spindle poles, it seems rather likely that the mechanism causing the movement on the spindle is closely related in the two cases. (This need not involve any assumptions concerning the nature of the mobile particle of the end and the centromere; they may or may not be related in structure.)

This introduction may be ended by mentioning some cases from other materials, which may be related to this case of mobile ends in rye. RHOADES and VILKOMERSON (1942) have found something quite similar in maize. Plants homozygous for an abnormal chromosome 10 manifest »secondary kinetochores» close to the chromosome ends. These are attached to the spindle and move poleward during anaphase in advance of the normal centromeres. (We regret that their paper has not yet been available here in original; we have only seen an abstract of it.) An active attraction of chromosome ends to a cell organ that is closely related to a spindle pole, viz. the centrosome, has already been known for a long time in the case of the polarization of chromosomes (bouquet stage) during the early stages of meiosis in many animals. A case of this phenomenon, which in some respects is of special interest, has been recently described by HUGHES-SCHRADER (1943). In some mantids she found a double polarization. First there is a normal formation of a bouquet in leptotene. This is followed by an unoriented stage, whereupon a new polarization stage comes from mid-pachytene until the nuclear membrane is dissolved. During this second polarization the centrosome has divided and the two daughter centrosomes have often separated to opposite sides of the nucleus. Consequently the chromosome ends are now polarized in two opposite directions. This constitutes a definite demonstration that a real, active movement of the ends is involved.

SPINDLE STRUCTURE.

Forces of attraction that increase in strength with increasing distance are not known in the case of any actions operating through a vacuum, such as electric and magnetic phenomena. Consequently there is every reason to think that the mobility of centromeres on the spindle is closely linked up with the *material structure* of their surrounding medium, i. e. the spindle. Although the important structural features of the mechanism may to a large extent be submicroscopical and impossible to investigate, it is possible that some of them are reflected in

a visible manner in the microscopic image. A study of the structural details of the spindle is consequently of great importance, when the behaviour of centromeres and T-ends are compared.

Although the fibrillar appearance of the spindle, which is so characteristic of many fixed preparations, may largely be an artefact, this appearance *cannot be regarded as insignificant*, and it may well be able to give important information concerning the properties of the living spindle. Even if a living spindle does not contain such a pronounced fibrillation as many fixed spindles, it is obvious that a material substrate corresponding to this fibrillar structure already exists in the living cell. This is demonstrated, e. g., by the centrifuging experiments of SCHRADER (1934) and by the observations of SCHMIDT (1939) on the double refraction of the living spindle. There has been a great deal of discussion concerning »the reality of spindle fibres»; it may be sufficient here to refer to the reviews of SCHRADER (1944) and CORNMAN (1944). It has often been argued that they are invisible in living cells. Recently, however, spindle fibres have been seen even in living cells on favourable material (COOPER, 1941).

The fibrillar structures of the spindle have often been classified into different groups. Thus, SCHRADER (1932, 1934, 1944) speaks of

(1) »half spindle components» = »chromosomal fibres»,

(2) »continuous fibres», and

(3) »interzonal fibres» = »interzonal connections».

The *chromosomal fibres* form one of the most conspicuous differentiations in the spindle. In favourable materials they may be seen as fibre bundles connecting the tiny granules called »spindle spherules» (important parts of the centromere) with the poles (SCHRADER, 1944). These chromosomal fibres have by many authors been regarded as intimately involved in chromosomal movement as traction fibres for the chromosomes (SCHRADER, 1944). CORNMAN (1944), in his fine review, considers that the bulk of evidence strongly favours the traction fibre theory. (The »interzonal fibres» connect the two separating anaphase chromosomes, and are considered by SCHRADER, 1932 and 1944, to be composed of a kind of chromosome substance, and the »continuous fibres» are the rest of the spindle, fibres which sometimes appear to connect pole with pole.)

According to BERNAL (1940; cited from BARBER and CALLAN, 1943), the spindle is a tactoid structure formed by fibre molecules. He is basing his hypothesis of chromosome movements on this assumption, suggesting that an important rôle is played by »negative tactoids» formed by non-fibrous substance within the spindle.

BARBER and CALLAN (1943) extend the hypothesis of BERNAL by assuming that ». . . prior to metaphase, the surface of the centromere resembles that of the centrosome in that fibre molecules set perpendicularly to it» (l. c., p. 269).

ÖSTERGREN (unpublished) agrees with BARBER and CALLAN in assuming that the spindle particles set perpendicularly to the centromere or, as he rather thinks, to a specialized part of the centromere, the »spindle spherule» of SCHRADER. He is changing the hypothesis, however, by assuming that the orientation of particles at the centromere persists until the end of anaphase and that this orientation brings about a condensation of spindle substance, which is visibly manifested as the »chromosomal fibre». Thus the chromosomal fibres are considered by him to consist of the same material as the rest of the spindle and to be in dynamic equilibrium with this rest, i. e. they would continuously exchange particles with the rest of the spindle. By orienting the particles like this (which must be a disturbance of their spontaneous equilibrium), the centromeres would give rise to forces which are the active factors of chromosome movements. The chromosomal fibres are assumed to be a kind of liquid crystalline »traction fibre», shortening or stretching by losing substance to or gaining it from the rest of the spindle. It is furthermore assumed that the spindle may get a sufficient rigidity by the presence of *reversible* fixing points (»Haftpunkte») between its particles, as assumed by FREY-WYSSLING (1938) for the cytoplasm in general. By their reversibility these fixing points will render possible that mobility of the particles which is a necessary prerequisite in any tactoid theory.

However it may be with this assumption, when it is considered that the specific property of active mobility of the centromere must largely depend on the material organization of the spindle (a point especially emphasized also by CORNMAN, l. c.), it seems extremely likely that this characteristic structural differentiation of the spindle (the chromosomal fibre), which is seen to attach to the centromere, should be the visible manifestation of a submicroscopical »machinery» responsible for the centromere movements on the spindle.

MATERIAL AND METHODS.

The structure of the spindle is well seen in chrom-acetic-formalin fixed material, sectioned on a microtome and stained with iron acetocarmine (the method of EHRENBERG and ÖSTERGREN, 1942). It is possible also to use old preparations previously stained with gentian violet. The cover-glass is removed by xylol + alcohol, the slide is passed through a decreasing series of alcohols to water and is put overnight in iron acetocarmine, it is washed in water and 45 % acetic acid and passed through increasing alcohols to xylol and mounted as usual. It was found by ÖSTERGREN (unpublished) that if an acetocarmine solution with only a low iron content was used it was possible to apply

an ordinary crystal violet stain after the acetocarmine and thus get a double stained slide with red spindles and violet chromosomes.

Some of the slides of PRAKKEN and MÜNTZING (1942) were restained by these two methods, and they form the material of the present study. The slides partly belong to the high intensity line No. 129, which contains two homologous T-chromosomes forming a bivalent, and partly to the triploid plant found in this line, which contains three T-chromosomes pairing as a trivalent or as a bivalent plus a univalent as is usually the case in autotriploids.

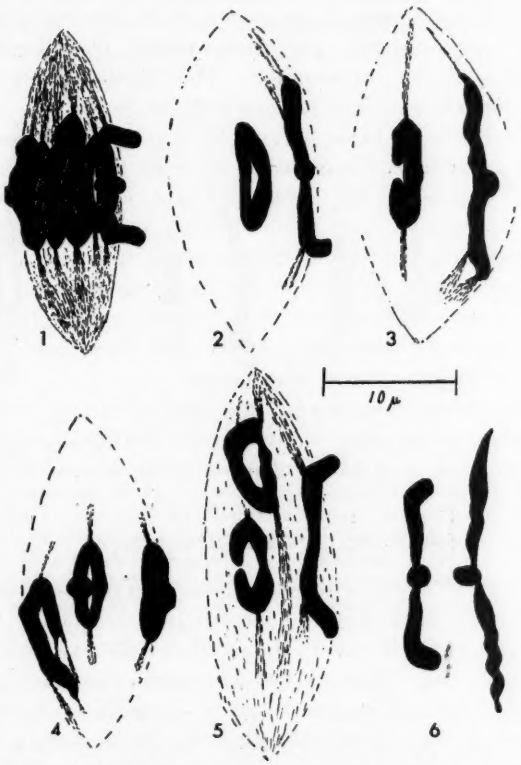
T-ENDS AND SPINDLE FIBRES.

The T-effect is variable in its manifestation, in some cells at first metaphase no active T-ends can be seen. In Fig. 1 a cell with no visible T-ends is drawn, to demonstrate the appearance of the spindle. Attached to all the centromeres can be seen characteristic spindle differentiations appearing like bundles of very fine fibrillae — the »chromosomal fibres». They start from the centromeres and are directed towards the poles, but often they cannot be followed distinctly all the way to the pole. They are most distinct close to the centromeres and their outline gradually becomes more and more indistinct in regions more distant from the centromere. Close to the centromere they appear more dense and narrow (»concentrated», so as to say) and away from the centromere they are thinner and broader (»dilute»). Close to the pole they again get narrower, if they can be distinguished at all from each other. In the regions distant from the centromeres they may, as it appears, not be well demarcated from each other, but are more or less »confluent».

As is well known, the spindle is highly sensitive to differences in fixation; so it is not astonishing to find that the chromosomal fibres may be somewhat variable in appearance and that sometimes they cannot be distinguished, a variation which is, of course, also conditioned by such circumstances as their small size (difficulties of observation) and by variations in the success of the staining as well as the possible presence of thick layers of stained cytoplasm below and above the structures to be observed, etc. This variation should not be taken to show that the material substrate of these chromosomal fibres in the living cell would be rather variable in development, and that it might sometimes even be absent. Such a suggestion seems extremely unlikely; our observations have convinced us that this spindle structure is a

constant and characteristic formation developed in connection with the active centromeres, the variation being due to the technical difficulties.

Considering the similarity in behaviour between the centromeres and the T-ends, it was of great interest to see whether the active T-ends also were furnished with similar fibre bundles. And, indeed, in many cases it could be quite clearly seen that such »chromosomal fibres» or



Figs. 1—5. The fibre bundles of spindle substance at centromeres and T-ends. — Fig. 1, bivalents and spindle in a cell showing no T-effect. — Figs. 2—5, bivalents involving the T-chromosomes. The position of the metaphase plate is indicated by drawing one or two of the normal bivalents on the same spindle. Note the displacement of the T-bivalents in Figs. 4 and 5. A schematical representation of Fig. 4 is given in Fig. 11.

Fig. 6. Comparison of a rod bivalent showing T-effect with a normal rod bivalent from the same cell. From the elastic modification of shape in the two cases it seems reasonable to conclude that the forces working at the T-ends are well comparable in magnitude with the centromere forces. — × 2000.

»half spindle components» are attached also to these ends. Some cells with T-bivalents showing this are depicted in Figs. 2—5. In Fig. 2 bundles are seen at the two centromeres and at one end, the other end being, as it seems, inactive. The figure is thus also an example of the commonly occurring variability in the manifestation of the T-effect. In Fig. 3 a rod bivalent shows chromosomal fibres at both ends and at one centromere. In the rod bivalent of Fig. 4 bundles of spindle fibres are visible at the two centromeres as well as at the two ends. At one of its ends there are, indeed, two fibre bundles, corresponding to the two chromatids of the chromosome. This bivalent is bent and displaced in a characteristic way because both its two active chromosome ends are directed to the same pole. Fig. 5 is a similar case of a ring bivalent, the active ends are probably involved in a terminal chiasma.

The fibre bundles of the T-ends are as a rule narrower and more difficult to see than those of the centromeres. As the technical difficulties sometimes make the observation of the spindle fibres of centromeres impossible, it is only to be expected that the same difficulties will often prevent the observation of the corresponding structure of the T-ends. It cannot be doubted, however, that these fibre bundles are a characteristic feature of the active ends.

Of course, these observations that the T-ends are modifying the spindle structure in the same manner as the centromeres give a strong support to the view that the mechanism of movement is the same in both cases.

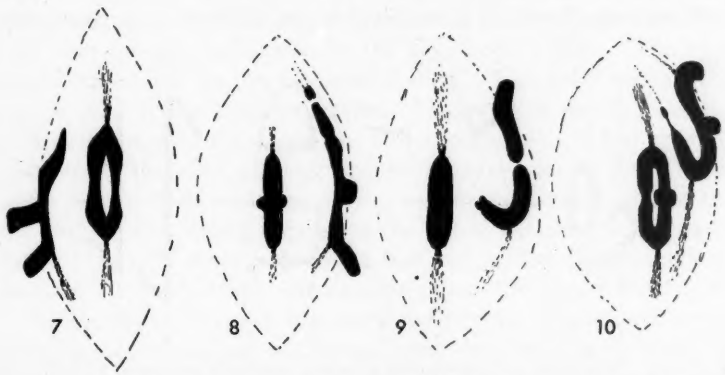
FORCES AND EQUILIBRIA.

The T-ends of bivalents and univalents are often seen at metaphase to subject the chromosome arms, which they terminate, to a strong tension. This tension definitely demonstrates the existence of a force working at the end. The elastic deformation of the chromosomes caused by this tension may be seen from many of our figures. A strongly pronounced case is depicted in Fig. 6, showing a normal rod bivalent and a T-rod from the same cell. The deformation at the ends of the T-rod is comparable in size to the deformation of the chromosome part extended between the two centromeres in a normal bivalent. From such pictures it can be concluded that T-ends are subjected to forces that are often similar in magnitude to those working at centromeres.

Undoubtedly, the metaphase position of normal bivalents must be an equilibrium, where forces working in various directions are

balanced. From the shape of the bivalents the existence of forces pulling at the centromeres is obvious. From the position of trivalents at metaphase it is clear (ÖSTERGREN, 1945 and unpublished) that these forces are best described as attractions to the poles, attractions growing stronger with increasing distance. Finally, the fact that the bivalents are so often found in a regular metaphase plate in fixed cells clearly demonstrates that this stage is of relatively long duration.

At metaphase the T-bivalents, besides being exposed to the special forces of the T-ends, are no doubt also subjected to the same centro-



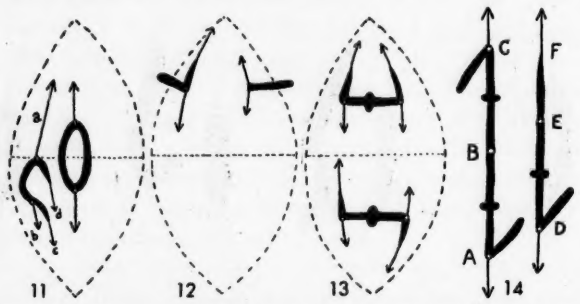
Figs. 7—10. Some cases of »asymmetric» T-rod bivalents, i. e. such having one active and one inactive T-end. — In Figs. 7—8 the active end is directed to the same pole as the centromere of the chromosome, and in Figs. 9—10 the end is directed to the opposite pole. Figs. 9—10 show that most probably the difference in activity is not caused (as otherwise might be suggested) by the active end being closer to a pole than the inactive. Note that the active ends are turned inward (towards the spindle axis) in Figs. 9—10. — × 2000.

mere forces as work on other bivalents. These latter forces will tend to keep them in the equator. The T-ends will often, e. g. Fig. 4, try to move them out of this position. We should expect the T-bivalents sooner or later to settle down at a position where these different tendencies balance.

The time needed by the normal bivalents to find their metaphase equilibrium is called the prometaphase (metakinesis). Prometaphase has ended and metaphase started when the last bivalent has reached the metaphase plate. Will prometaphase be a sufficient time for the T-bivalents also to find their equilibria? This will depend on the velocity of movement of the T-ends. If they move rather slower than

the centromeres, prometaphase time will be insufficient, otherwise the differences will be of no practical importance. During the anaphase movements, the T-ends are found to move at least as rapidly as centromeres, as they do not tend to lag after the centromeres, but rather the contrary. Furthermore, as already mentioned, the similarity of spindle structure at T-ends and centromeres may suggest that the mechanism of movement is the same. Thus, most probably the prometaphase will suffice; and the metaphase positions of T-bivalents will represent equilibria. We have seen in our slides that the T-ends are already active at prometaphase.

When both T-ends of a rod bivalent are directed to the same pole,



Figs. 11—14. Schematic representations of some equilibria involving T-ends. The arrows indicate forces. — Fig. 11 corresponds to Fig. 4. The force a is able to balance the sum of $b + c + d$. (The tendency of the chromatin to move out of the spindle in a transverse direction has not been represented by arrows in these figures.) — Fig. 12, the two chief types of equilibria of T-univalents. In the left univalent the force at the T-end is counteracted by the force at the centromere, and in the right univalent the two chromatid ends of the same chromosome end are pulled in opposite directions. — Fig. 13, the equilibria where T-bivalents function mechanically as two T-univalents associated by a chiasma (two bivalents of different types drawn on the same spindle). There has been a co-orientation *within* each of the two chromosomes forming a bivalent, between T-end and centromere of the same chromosome. — Fig. 14. There may be some parallelism between asymmetric T-bivalents and certain trivalents. In a trivalent it may sometimes happen that two of the centromeres are oriented (*A* and *C*) while the third (*B*) lying between them may fail to orientate. Similar conditions may exist in a T-rod (*F* and *D* are oriented, and *E* is not). The tension between *F* and *D* may prevent *E* from orienting. In such a case the equilibrium would be determined by *F* and *D*, and *E* would be practically without effect. The position of asymmetric T-rods indicates that such a mechanism may sometimes work.

the bivalent is often strongly displaced from the metaphase plate in that direction (Fig. 4). This is also the case of ring bivalents such as in Fig. 5. Here, too, most probably both ends have the same direction,

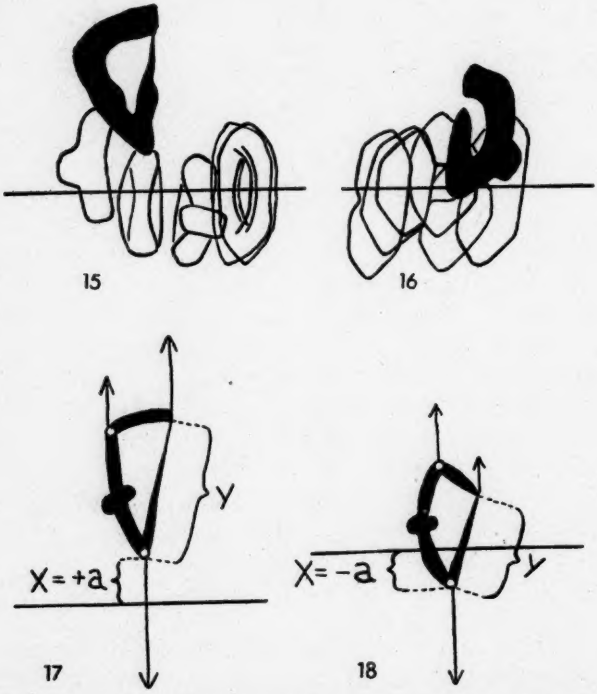
although this cannot be clearly seen because they are so closely associated. The forces working in such a case as Fig. 4 are represented diagrammatically in Fig. 11. As the force a in the figure will balance $b + c + d$, it is obvious that the poleward forces at the centromeres will increase in strength with increasing distance from the pole as suggested by ÖSTERGREN (1945 a).

Ring bivalents like those of Figs. 5, 15 and 16 will furnish one more possibility of testing this view concerning the forces. Such ring bivalents are not always displaced to the same extent, but their position is rather variable. If the above hypothesis is true, their variable degree of displacement should be due to a variation in the size of the displacing force located at the associated T-ends. The short chromosome arm y in the schematical Figs. 17 and 18 will have to carry the load of this displacing force. Obviously its elastic distension should be stronger the stronger the T-end force is; i. e. a stronger displacement of the ring bivalent should be accompanied by a more strongly extended chromosome arm y. A variation in the size of this T-end force is very likely because of the strong variation in the manifestation of the T-effect found in the material (giving sometimes, e. g., asymmetrical rod bivalents with only one active end; Figs. 2 and 7—10).

The correlation between the elastic deformation of the bivalent and its displacement from the metaphase plate was statistically tested in the following way. A microtome section of a loculus containing a high frequency of such T-rings was selected. The bivalents were drawn by means of a camera lucida in the natural positions which they had in the cells. Two such figures are given in Figs. 15 and 16. From the position of the normal bivalents the plane of the metaphase plate was estimated and drawn in the figures. Figs. 17 and 18 demonstrate the distances measured on the drawings; y is the length of the distended chromosome arm and x is the distance of the lowest centromere to the equator. The figures also demonstrate under what circumstances x is defined as positive and under what it is negative. (Thus a negative x should not be mistaken for a displacement of the ring in a direction opposite to the force acting on the T-end. No such rings have ever been seen.) The values of our measurements (in units) are as follows (y; x): (21; 3) (21; —2) (23; 9) (20; 6) (21; 3) (27; 8) (25; 13) (23; 7) (27; 1) (22; 7) (25; 1) (25; —4) (23; 2) (23; 5) (32; 12) (22; —6) (21; 6) (22; 3) (19; —3) (19; —1) (23; 3) (18; —7) (28; 7) (19; —6) (16; —3) (21; 4) (25; 6). By analysis of co-variance it is found that the probability for independent variation of x and y is

p = ca. 0,001. In a material of measurements chiefly made on the same cells as the above but also including some others from somewhat dubious cells, the p value was between 0,01 and 0,001. So the measurements give a fairly good support to the impression that there is such a correlation, a fact which strongly supports the above considerations concerning forces and equilibria of T-bivalents.

The displacement of the T-bivalents strongly simulates that of the trivalents observed by ÖSTERGREN (1945) and BÖÖK (1945). The degree



Figs. 15—18 illustrate the method of measuring employed in the effort to correlate the displacement of T-ring bivalents with their degree of elastic modification of shape. Figs. 15—16 show two actual cases, and Figs. 17—18 show schematical interpretations. The position of the equator is estimated from the position of the normal bivalents. A numerical value for the position of the T-ring is obtained by measuring (on a camera lucida drawing) the distance x between the equator and that centromere which is oriented in a direction opposite to the T-ends. The distance y is the length of the short chromosome arm, which is extended between its centromere and its T-end. Figs. 17—18 also demonstrate under what circumstances x is defined as positive or negative. — Figs. 15—16, × 4000.

of displacement is often of a similar order, which is further evidence for the view that the T-end forces are often of the same order of magnitude as the centromere forces. Undoubtedly the two displacement effects are closely related. It may be said that although the T-bivalents are bivalents from a genetical point of view, they are multivalents from a mechanical point of view.

T-rods active at one end only (Figs. 7 and 8) would be expected to be displaced in the direction in which this single end is pulling. Although this may happen, it also happens, strangely enough, that they are not displaced at all or are even displaced in the »wrong» direction. It may, however, be possible to explain this without making too complicated assumptions. The forces working at centromeres (and T-ends) are highly dependent on the *orientation* of the particle in question, as pointed out by ÖSTERGREN (1945 a and b). This particle can be attracted only by that pole towards which it is oriented. (The questions associated with the orientation and co-orientation of centromeres are undoubtedly very complicated and but little understood.) Now, from a mechanical point of view, a T-rod of the type of Fig. 7 is a trivalent. One of the configurations which trivalents may sometimes adopt at metaphase is that given in Fig. 14 (the left figure). It may occur that two of the chromosomes, *A* and *C*, are oriented to each of the two poles, while the middle one, *B*, is occupying an unoriented »neutral» position. Probably the tension between the centromeres *A* and *C* can prevent *B* from orienting. Similarly, it is close at hand to suggest that the tension between the T-end, *F*, and the centromere, *D* (in 14, right figure), may prevent *E* from orienting. Under such circumstances the position of the configuration will depend on the relative strengths of *D* and *F*, and variations in the intensity of the T-effect between bivalents will cause some of them to be displaced in one direction and others in another.

What is the cause of the variability in the intensity of the T-phenomenon referred to on some occasions above? This variation occurs in a homozygous long-inbred strain. The cause of asymmetric bivalents like those in Figs. 7—10 can scarcely be heterozygosity, because cells containing them are intermixed at random among many cells having T-bivalents with an equal activity in both chromosomes. Probably the cause is that the activity of the T-ends is very dependent on a highly specialized physiological condition in the cell. Small deviations from this condition may strongly affect the manifestation of the phenomenon. A support for this view is the fact that the T-effect

is manifested *only during the two divisions of meiosis* (PRAKKEN and MÜNTZING, 1942) and not in the ordinary mitotic divisions. In this respect there is an important difference between the properties of T-ends and centromeres. The importance of the orientation of the particles for their behaviour has already been mentioned. A possible cause of variable T-effect might also be a tendency of the T-ends to have difficulties of orientation.

The material studied also contains a triploid plant having T-effect. In this plant, besides bivalents with T-effect, there also occur trivalents and univalents that show such an effect. The T-trivalents offer nothing of interest which is not already known from the bivalents, but they are more complicated (PRAKKEN and MÜNTZING, l. c., Figs. 98—102). The univalents, however, may be worthy of a special description.

Normal univalents in this triploid plant lie distributed rather at random over the surface of the spindle and their centromeres can sometimes be seen to be furnished with spindle fibres as in Fig. 19. The random distribution may largely be a matter of unsuccessful centromere orientation (ÖSTERGREN, unpublished). (A more detailed description and discussion of the behaviour of normal univalents is outside the scope of the present paper.)

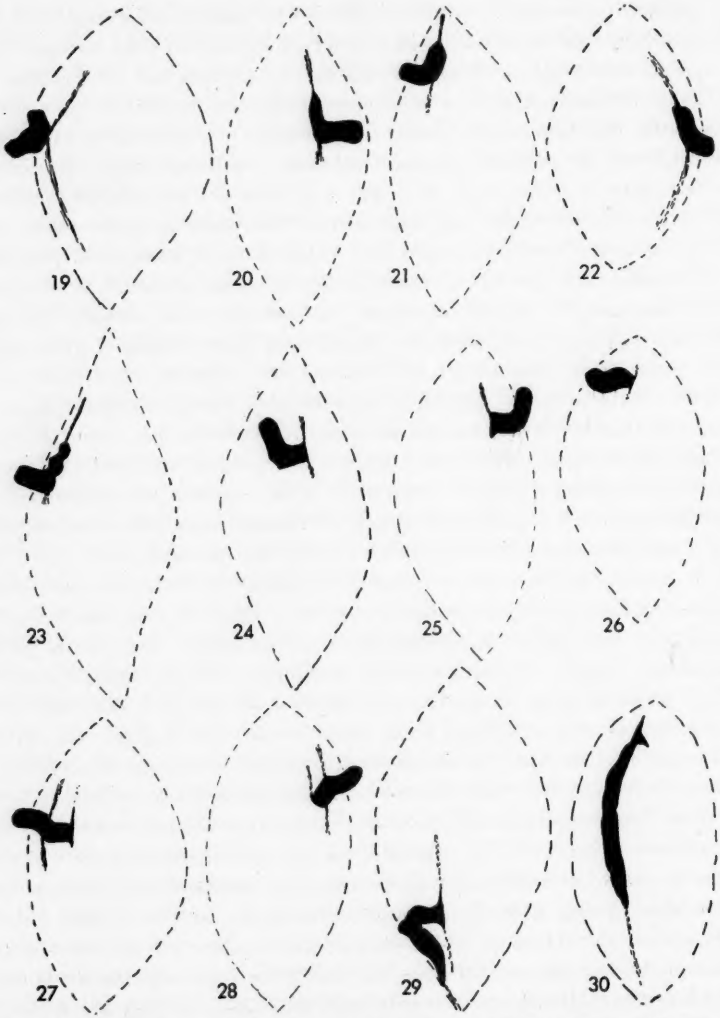
The T-univalents can very often be seen to have their T-end directed towards one pole and their centromere towards the other. The chromosome arm between the centromere and the T-end is directed lengthwise in the spindle and can be seen to be subjected to tension (Figs. 20—23). This demonstrates that there are forces working in opposite directions at the end and at the centromere. The tension cannot be due, as might be suggested, simply to a rapid poleward movement of the T-end dragging the chromosome through a viscous medium. The absence of movement is demonstrated by the direction of the inactive chromosome arm of the T-univalents. This arm is not directed in the lagging manner characteristic of anaphase movements, but it is often pointing in a direction perpendicular to the axis of the spindle. The positions of the T-univalents at metaphase obviously represent equilibrated positions which they have most probably reached during the prometaphase movements. Sometimes characteristic fibre bundles of spindle substance, »chromosomal fibres», can be seen to attach both to the T-end and to the centromere of these univalents and to proceed from the end towards one pole and from the centromere to the other pole.

The process which makes the two centromeres of a bivalent orient

in opposite directions on the spindle is called co-orientation by DARLINGTON (1937). At least in a descriptive sense it may be said that in such T-univalents as Figs. 20—23 the T-end and the centromere of the same chromosome have co-oriented relatively to one another. The univalents function mechanically as bivalents. They differ from bivalents, however, in one important manner. Although their metaphase position may be in the plate (Fig. 22), it is in most cases situated outside the plate, displaced more or less towards that pole towards which the end is directed (Figs. 20, 21 and 23). This variable metaphase position is most probably one more effect of the variable manifestation of the T-phenomenon to which reference has already been made. Strong T-ends would obviously shift the equilibrium more closely to their pole than weak ones. An effort to correlate the position of such T-univalents with the elastic distension of their short arms, similarly as was done with the bivalents, was unsuccessful. None the less, there may of course exist such a correlation. The failure may be explicable if there are in these univalents also variations in the success of orientation of centromeres. The co-orientation in T-univalents may not be so regular as between the two centromeres of normal bivalents.

It would also be expected that T-univalents of the type mentioned might sometimes occupy metaphase positions closer to the pole towards which the centromere is directed than to the other, viz. in the cases where the activity of the T-end is especially weak, so that it can be pulled very far away from the pole towards which it is directed. No clear cases of this kind have been found, however. A good reason for this is the fact that in chromosomes where the T-activity is very weak it must be connected with difficulties to recognize its existence.

The T-univalents can also behave in another characteristic way on the spindle (Figs. 24—27). In this case the two chromatid ends of the same chromosome end with T-properties have oriented towards opposite poles, thus giving a kind of »auto-orientation» at the T-end. Such ends are characterized by (1) a characteristic shape due to the tension between the two chromatid ends, (2) the often seen attachment of two fibre bundles to them, one towards each pole, and (3) they are directed inward towards the axis of the spindle (cf. below). The same process may also occur within chromosomes paired as bivalents (Figs. 29 and 30). Fig. 28 represents a more complicated case of a univalent where the centromere and one chromatid end are directed towards one pole and the other chromatid end is directed towards the other. The univalent »functions as a trivalent».



Figs. 19—28. The appearance of univalents in a triploid plant with the T-phenomenon. — Fig. 19, a normal univalent, not showing T-effect. Such univalents are lying on the surface of the spindle, and sometimes show fibre structures from their centromere towards both poles. — Figs. 20—28, T-univalents (compare Fig. 12). — Figs. 20—23 are of the type where the T-end and the centromere work in opposite directions, the end is co-oriented relatively to the centromere. Note fibre bundle present at both the end and the centromere in Figs. 20 and 22, and the drawn-out chromatin threads at the T-ends in Figs. 21 and 23. — Figs. 24—26, the

In the bivalents, instead of the normal co-orientation between the two centromeres, there may also occur an alternative process. Thus, within each of the paired chromosomes there occurs a co-orientation of its T-end and its centromere relatively to one another. In this way configurations result like those schematically given in Fig. 13. The process of co-orientation is intrachromosomal instead of interchromosomal. T-rods of this type are found in Figs. 33—39. The bivalents behave as two T-univalents associated by a chiasma. A related T-ring is depicted in Fig. 40, where the two T-ends in a terminal chiasma pull in one direction and both centromeres in the other. It may also occur as in Figs. 31 and 32 that an intrachromosomal co-orientation is found only in one of the two partners of a bivalent, while the other is floating in a passive manner on the surface of the spindle like a normal univalent.

The forces considered in the above discussion of the equilibria of bivalents and univalents have been such as work chiefly in the longitudinal direction of the spindle, i. e. attractions to the poles of centromeres and T-ends. As pointed out by ÖSTERGREN (1945 b), the chromosomes are, however, also influenced by forces which work largely in the transverse direction of the spindle. Thus, the poleward attractions of centromeres will be also manifested as a tendency of them to move towards the centre of the metaphase plate. ÖSTERGREN (l. c.) also suggested that the chromosomes (except the centromeres) had a tendency to move out of the spindle because of the disturbance which they caused in the arrangement of the tactoid particles. The strong longitudinal centromere forces would keep the centromeres in the equator; and the chief effect is supposed to be a tendency of chromosome arms to come out transversely. These antagonistic tendencies of the centromere and the rest of the chromosome, he suggested, would

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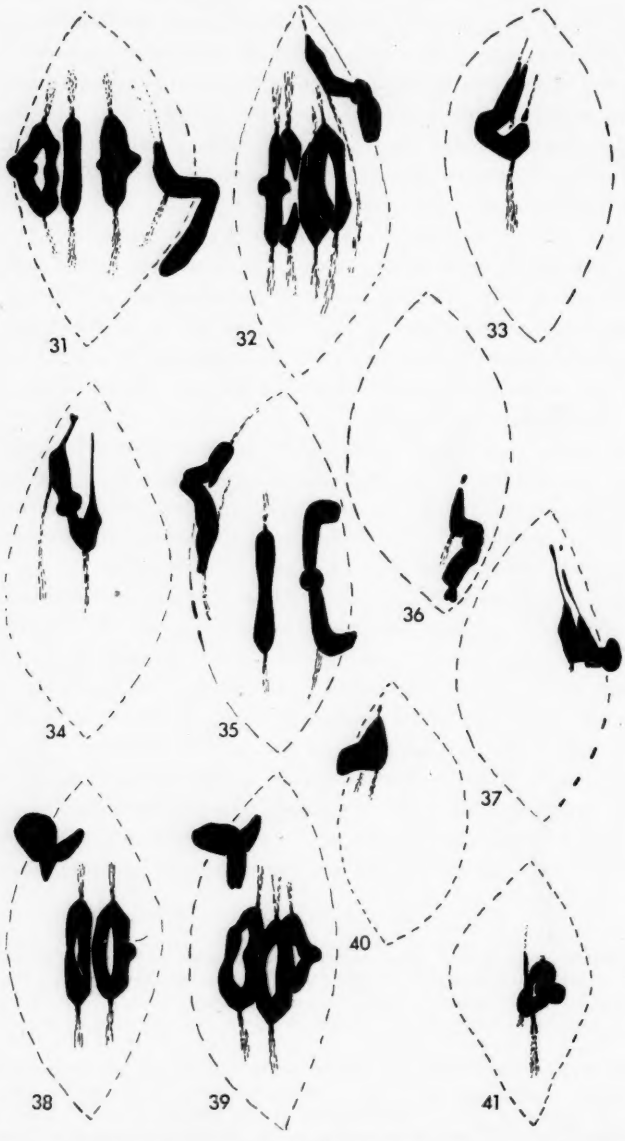
two chromatid ends of the same chromosome end pull in opposite directions, the centromere gives no evidence of activity (as is often so in univalents). — Fig. 27, the T-end behaves in the same way as in 26, but here fibres are also seen at the centromere. — Fig. 28, a related case where, however, the centromere fibres may be directed to one pole only.

Figs. 29—30. Bivalents where the two chromatid ends of the same chromosome end are directed towards opposite poles. — Figs. 24—30 are also of value in showing that a T-chromosome end can be attracted by the pole more distant from it instead of that closer to it. The decisive factor of the direction of attraction is obviously not *position* but *orientation*. — Note the common occurrence of the inward direction of the »active» parts (centromeres and T-ends) and the outward tendency of the »inactive» chromosome parts. — × 2000.



Figs. 31—41. Co-orientation between centromere and T-end of the same chromosome in bivalents (compare Fig. 13). — Figs. 31—32, only one of the two partners shows such an »intrachromosomal co-orientation», the other partner is floating in an

largely balance one another at metaphase, thus giving *transverse equilibria* of the chromosomes.

This view is supported by the arrangement of long chromosomes with a subterminal centromere. When these lie at the periphery of the metaphase plate of mitosis, they usually have their centromere in a central and their long chromosome arm in a more peripheral position on the plate. The present material supplies some more examples of this same phenomenon of transverse equilibria. Thus, in the T-univalents (Figs. 20—28) it is a very characteristic feature that the active components (T-ends and centromeres) are oriented inward towards the axis of the spindle while the inactive chromosome arms tend to point in a peripheral direction. This is easily interpreted as due to the mechanism suggested. The observation that the same inward tendency is working on both centromeres and T-ends is one more support for the view of their close relation in a spindle mechanical respect. It is furthermore a definite proof that both of them are *attracted* to spindle poles. Similar transverse equilibria are also of common occurrence in the bivalents. The inward tendency of the active parts combined with the outward tendency of the inactive is seen, e. g., in our Figs. 9, 10, 31, 32, 33, 35, 36, 37, 38, 39, and 40.

Fig. 41 represents a configuration which is worth discussing with a few words. It is a ring bivalent lying in an equatorial position. The chromosome arms with the T-ends are involved in a subterminal or perhaps terminal chiasma. The T-ends are strongly attracted towards the two opposite poles, so that the chromatin close to them is distended to threads. To these ends it can be seen that spindle fibres are attached. The equatorial position of the ring is obviously caused by a balance of the attractions working in the two opposite directions on the spindle. No such chromosome configuration had been observed by PRAKKEN and MÜNTZING in their study, although such bivalents were especially searched for, because their existence or non-existence was regarded as being of some theoretical importance. From the present study it is

indifferent way on the surface of the spindle just as univalents usually do. — Figs. 33—40 show intrachromosomal co-orientation in both components of the bivalents. — Figs. 33—35 and 37 correspond to the upper bivalent in Fig. 13, and Figs. 36 and 38—39 correspond to the lower one. — Fig. 40 shows a ring bivalent related to these rod bivalents; it has a (probably terminal) chiasma at the active chromosome ends. — Fig. 41 shows a ring bivalent having active T-ends directed towards both poles. It is not displaced from its equatorial position. — The centripetal tendencies of the active chromosome parts and the centrifugal tendencies of the inactive ones are also visible in most of these figures. — × 2000.

obvious that they are found only very rarely, although they doubtless do exist. One reason they are so rarely observed is indicated by the existence of transverse equilibria of the type mentioned above. Such rings are attracted poleward both by a pair of centromeres and by a pair of T-ends; so they should manifest an especially strong tendency to lie in a central position in the equatorial plate. In such a position there is a very good chance that the thin extended T-ends will be concealed from observation by the surrounding normal bivalents. Perhaps this explanation alone is not sufficient, however. We are inclined to believe that some additional cause of their rare occurrence must be looked for, and we suggest that there might be special properties of a terminal chiasma of T-ends, which often make the ends function as a unit in orientation, and thus prevent them from turning towards opposite poles.

The present interpretations of forces and equilibria differ in some respects from those given by PRAKKEN and MÜNTZING (1942). The chief reason for this is that in this previous paper these problems occupied a relatively peripheral position and were therefore not analysed so profoundly. Thus, starting from the normal type of displaced T-rings, it was suggested at that time that the T-ends were attracted by the pole closest to them and that the attraction might grow stronger with decreasing distance to the pole. The fact that no configurations of the type depicted in our Fig. 41 had been found in the previous study was regarded to support this view; the absence was interpreted as being due to a balance of the influences from the two opposite poles on T-ends lying at the same distance from both, in the equator. At present it is believed that T-ends are attracted towards the pole towards which they are *oriented*, independently of whether this is the nearest pole or not. In the following figures of the present paper it can be seen that T-ends are attracted by the most distant pole instead of the one closest to them, viz. Figs. 24, 25, 26, 27, 28, 29, 30, 35, 36, 38, 39. Obviously, however, T-ends will tend to move towards the pole towards which they are attracted, and for this reason they will very often be found to be directed towards the pole closest to them, a fact which may easily lead to a misunderstanding of the type mentioned.

In the study of PRAKKEN and MÜNTZING some cases were also recorded in which it was believed that a chromosome end, which during the anaphase movement approached the pole it had previously been directed away from, under such circumstances came under the influence of the new pole and turned around towards it. Perhaps such figures as 55 and 56 by PRAKKEN and MÜNTZING might instead be interpreted otherwise, however. It is possible that the »turned around» appearance is caused, instead, by a slowing up of the movement of the end close to the pole, leading to a bending of the chromosome arm when the centromere is approaching the end. As, however, the knowledge of the factors determining the orientation of ends is very little known, the possibility is still open that a *reorientation* may really occur, which, of course, would then be followed by an attraction to the new pole.

SUMMARY AND CONCLUSIONS.

The actively mobile chromosome ends (T-ends) of some inbred strains of rye previously studied by PRAKKEN and MÜNTZING (1942) have been re-investigated with other methods. The T-ends turned out to be furnished with similar spindle fibre bundles (»chromosomal fibres») as are attached to the centromeres. The equilibria of T-bivalents and T-univalents at metaphase strongly support the view that T-ends and centromeres have the same mechanism of movement on the spindle, and that they, in accordance with the view of ÖSTERGREN (1945 a and b and unpublished), are attracted to spindle poles by forces increasing in strength with increasing distance.

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MEIOSIS IN HYBRID NULLISOMICS AND CERTAIN OTHER FORMS OF GODETIA WHITNEYI

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MONOSOMICS showing unreduced vitality but a rather pronounced pollen sterility are known in *Godetia Whitneyi*. Dr. GUNNAR HIORTH has collected seeds from wild-growing plants of this species, and monosomics have been found to occur in several localities. The cytology of monosomics from Briceland, Horse Mtn., Santa Rosa 6 and Tocaloma has been studied (HÅKANSSON, 1945; concerning the localities compare HIORTH, 1942). The results of the genetic studies of HIORTH have, however, not yet been published. The monosomics are structural hybrids, their chromosome configuration at metaphase 1 is 4 II + I + + chain-of-four. Only in Briceland the configuration of four chromosomes was rather often a closed ring, other monosomics always had a chain. The chromosomes of the chain are zigzag arranged and adjacent chromosomes pass to different poles. Very often the univalent is eliminated because it is lagging at the equatorial plate. The chromosomes of the chain in Tocaloma have been designated 4'.1 — 1.2 — — 2.3 — 3.4, the chromosome 1.4' is large, the segment designated 4' cannot form a chiasma, the other end-chromosome 3.4 is small. The univalent has been designated 5.6. The monosomics may be considered as heterozygous complex combinations, *a.g*; *a* is the normal or standard complex of *G. Whitneyi*, *g* is a complex with the changed chromosomes 1.4' and 2.3 but lacking chromosome 5.6, thus, *g* has only 6 chromosomes. Cytogenetic investigations have shown that functioning gametes are *g*, *g* + 5.6 or *a*; *a* gametes with 6 chromosomes (lacking 5.6) never function. Owing to the frequent elimination of the univalent the great majority of the functioning embryo-sacs and pollen-grains of the monosomics have 6 chromosomes and are *g* gones. Cytologic and genetic observations support the view that portions of the chromosome 5.6, which is not a regular member of the *g* complex, must be present in the *g* chromosomes in the chain, probably in the large chromosome 1.4', but perhaps also in 2.3. The forming of the *g* complex is the

result of a reciprocal translocation between one of the changed chromosomes in a ring and chromosome 5.6. Through this translocation an inert chromosome 5.6 was also formed that is lost.

The cytogenetics of the four monosomics are different, all are called *a.g* but the *g* complexes may show differences. The *g* complex in the monosomic from Briceland is here sometimes called g^1, in that from Horse Mtn. g^2, from Santa Rosa 6 g^3, and from Tocaloma g^4. The monosomics $a.g^1$ and $a.g^4$ could be studied by HIORTH very thoroughly, because g^1 has a dominant gene C^{st}, and g^4 another dominant C^{pa}, both causing certain musters on the cotyledons. Their loci are in one of the *g* chromosomes of the chain. The cytogenetics of the different monosomics may be briefly summarized thus: (1) $a.g^1$ is, as it seems, a constant monosomic, crosses with *a.a* (the garden race Bremen) of the type $C^{st} \times cc$ have given C^{st} (monosomics) and only 2 plants of 1108 were *cc*; in the reciprocal cross *cc* ♀ × C^{st} ♂ there was, however, 25 % *cc*. (2) $a.g^2$ segregates *a.a*, $a.g^2$, $a.(g^2 + 1)$, with the pairing 5 II + chain-of-four, and $g^2.(g^2 + 1)$ with the pairing 13 I. The type last mentioned is very sterile. (3) $a.g^3$ segregates *a.a*, $a.g^3$, $a.(g^3 + 1)$ and $g^3.g^3$. This last type is dwarfish, has small flowers and is very sterile, and the pairing is 12 I. (4) $a.g^4$ segregates *a.a*, $a.g^4$, $a.(g^4 + 1)$. Non-disjunction in the chain and interchanges are known to occur and add to the variability of the monosomics.

I have now been able to investigate plants that have been produced by crossing different monosomics, and also some plants produced by crossing the nullisomics from Santa Rosa with other types. The material was fixed in Ås in 1944; in 1945 no fixations could be made. Some hybrid combinations have more or less deformed flowers with reduced anthers, in some cases the flower bud had only one or two small loculi with a few p.m.c's. This made meiotic studies difficult.

SANTA ROSA 6.

This is the only monosomic that segregates a nullisomic. The nullisomic plants appear in great numbers owing to the excess of *g* gones. They show a very strong asyndesis, in a number of p.m.c's a restitution nucleus is formed, and about 28 % of the pollen sporads were diads. In the ovules macrospore diads were observed (HÅKANSSON, 1943). They were probably the result of the division of an e.m.c. with a restitution nucleus. Thus, egg-cells with 12 chromosomes must be formed rather frequently. The nullisomic plants first observed seemed

to be quite sterile. Later, nullisomics with a certain though low degree of fertility have been found by HIORTH.

Tetraploid nullisomics. — Two plants that were the result of selfing of the nullisomic were investigated. The mother plant had appeared in the F_2 of a cross monosomic S. Rosa × Bremen. One plant, 44—1305/832, had 24 chromosomes, a sister plant, 1304, had 25 chromo-

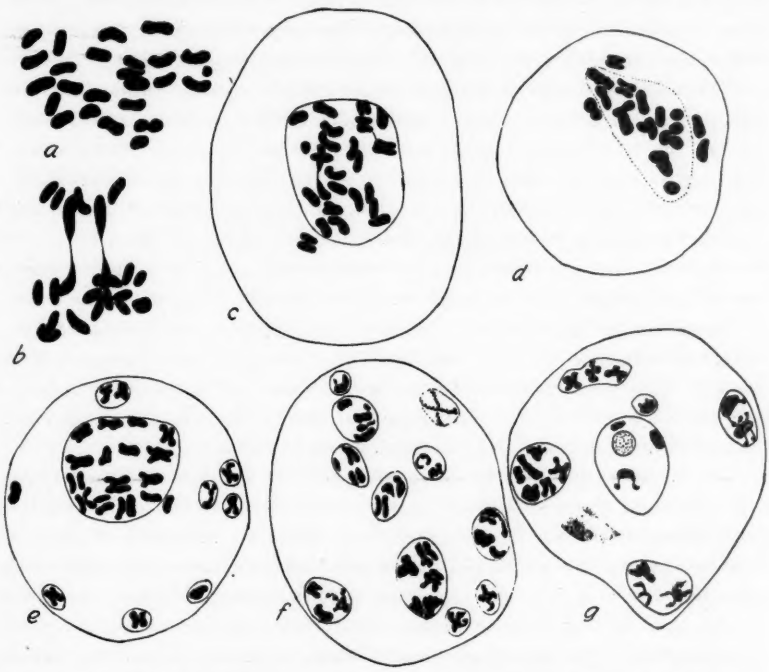

Fig. 1. Tetraploid nullisomics, *a* and *c* are from the hypertetraploid plant. *a*: 25 I. — *b*: two bivalents and univalents. — *c*: 1 II + 23 I. — *d*: the chromosome group now is near the wall of the p.m.c. — *e*—*g*: interkinesis; as a rule the nuclei are near the wall of the p.m.c.; this could not be shown in the figures.

somes, one of the chromosomes being a small fragment chromosome (Fig. 1 *a*).

The plants show a strong asyndesis, as a rule 24 or, in the hypertetraploid plant, 25 I are seen in the p.m.c. As in the diploid nullisomic one or rarely two II's may be formed. The stage following diakinesis is of long duration, the univalents form a more or less expanded group in the centre of the p.m.c. A regular equatorial plate including all

chromosomes was never observed, but no doubt a tendency to form a plate is manifest in many p.m.c's. A regular anaphasic distribution of the chromosomes to the poles through attraction fibres (see ÖSTERGREN, 1945) does not occur. Fig. 1 *b* looks like an anaphase with four of the I's directed to one pole, the rest towards the other, but this is probably not an anaphase 1, because the homologues of the two II's have not separated. However, certain movements of a small amplitude seem to be performed by the univalents. When the chromatids begin to be visible the univalents lie more or less dispersed.

At interkinesis the p.m.c's show a variable and sometimes curious appearance. Fig. 1 *e* shows eight small and one large interkinesis nucleus, the small nuclei all lie near the wall of the p.m.c., but also the large nucleus has an excentric position in the cell. One of the univalents was divided when the membrane of the large nucleus was formed, one of the chromatids is outside the membrane. As a rule, however, the centromeres are not divided before anaphase 2. Fig. 1 *f* shows many interkinesis nuclei; they were all near the wall of the p.m.c. (this could not be shown in the figure). Fig. 1 *g* also shows an interkinesis stage; at first glance one might be inclined to interpret it as a young pollen sporad. Rather often most of the chromosomes are included in a large interkinesis nucleus, but it is a fact that such a nucleus does not lie in the centre of the p.m.c., but nearer one end of the cell.

It is clear that the number and size of interkinesis nuclei are dependent on the distribution of the univalents in the cell when the membranes are formed. How are their different positions in the cell brought about? An anaphasic distribution of the chromosomes through attraction fibres that leads to the frequent assembling of most univalents at one pole of the spindle seems highly improbable. I have before (HÅKANSSON, 1940) mentioned some changes in the p.m.c. that occur at late anaphase 1 and early telophase 1. This was in a *Godetia* that very often had two univalents lagging in the equatorial plate. These univalents were then moved in a centrifugal direction to the wall of the p.m.c. The cause of this movement was apparently the forming of a kind of phragmoplast that expanded and finally reached the wall. During and after this expansion the centre of the phragmoplast seems to be transformed into ordinary cytoplasm. The process may perhaps be compared with the blowing up of a lemon-shaped ball, the spindle substance or phragmoplast being the wall of this ball, cytoplasm filling the interior of the ball. Univalents are attached to the phragmoplast and are therefore moved in centrifugal directions.

Transformations in the interior of the p.m.c. seem to occur also in the tetraploid nullisomic. The result is that rather isolated univalents or groups of univalents are spread out in centrifugal directions. But no doubt a larger group of univalents may also be moved or pushed as a whole. Fig. 1 *d* shows such a group that somewhat earlier was probably in the centre of the p.m.c. An increase in size of the p.m.c. often occurs at the same time. The univalents in the large group are held together, probably because the formation of a nucleus has begun and is in progress. A large interkinesis nucleus lying at one end of the p.m.c. was very often observed in an asyndetic form from Horse Mtn. (HÅKANSSON, 1943). This position had probably been brought about in the way here described.

Stages of the second division were only observed in 1305. They showed no unexpected traits.

Nullisomic × *disomic.* — The nullisomic had been pollinated with pollen from the garden race *flammea.*

Buds from eight plants had been fixed. One plant (835 f) was a monosomic, the pairing was 4 II + I + chain-of-four. Thus this plant was $a.g^3$. Sometimes the pairing was 6 II + I, and new I's could be observed, the plant showed a weak asyndesis. Seven plants were triploids, they had 19 chromosomes and were $a.g^3g^3$. In some triploids meiosis could be studied. At metaphase 1 tri-, bi-, and uni-valents were observed, at anaphase 1 lagging chromosomes are common.

It is a fact that the pairing in $a.g^3g^3$ is good. It is true that the number of trivalents per p.m.c. is lower than in the autotriploid *a.aa*, which has a very high frequency of III's (HÅKANSSON, 1940), but a comparison is more interesting with the pairing in $a.g^3$. This combination shows a weak asyndesis, which is also found in $a.(g^3 + 1)$, but is rather variable, and this is the reason the pairing in the triploids has been compared with that in their monosomic sister plant, in spite of the small number of p.m.c's that were well-fixed in the latter plant. As a measure of the degree of asyndesis in this monosomic plant the number of univalents was counted; if the p.m.c. had only one I there was no asyndesis. 19 p.m.c's had no asyndesis, 1 had two, 9 had three, 1 had four, 3 had five, and 1 had seven I. Thus, about 45 % of the p.m.c's had asyndesis. This is a higher percentage than in other monosomic plants from S. Rosa.

In the triploids the degree of asyndesis was not estimated by counting the I's. The reason is the somewhat labile pairing that leads to the surmise that a III is more often divided in II + I than a II in

I + I. Here the sum of the III's and II's was determined in the p.m.c. In plant 835 h 27 p.m.c's had 7, 12 had 6, and 4 had 5 trivalents and bivalents. In plant b 14 p.m.c's had 7, 11 had 6, 8 had 5, and 1 had 4 tri- and bi-valents. Thus, plant h had many more p.m.c's with the high number of seven III + II than with lower numbers. It seemed that in b the investigated p.m.c's were nearer the anaphase 1 than in h, and this probably explains the observed difference in pairing. Be that as it may, plant h cannot have a weaker pairing than the monosomic f and this means that the combination $a.g^3g^3$ does not cause a stronger asyndesis than the combination $a.g^3$. In view of the very strong asyndesis of $g^3.g^3$ this is of some interest.

This cross shows that the nullisomic can also form functional reduced egg-cells containing the *g* complex. Functioning unreduced egg-cells, however, were much more numerous.

Disomic × *nullisomic.* — Plants of the *Whitneyi* race C^a had been pollinated with pollen from a nullisomic. As in the cross last mentioned, the nullisomic plant was an F_2 from S. Rosa monosomic × Bremen. C^a has the standard chromosomes of *G. Whitneyi.*

Eight plants were fixed. 5 (836 a—e) were monosomics, 3 (f—h) were triploids with 19 chromosomes. The monosomics showed in most p.m.c's a certain asyndesis. The normal arrangement 4 II + I + chain-of-four could sometimes be seen. Other observed configurations may be mentioned: 4 II + 2 I + chain-of-three, 6 II + I, 4 II + 5 I. The asyndesis of monosomics and triploids was probably stronger than in the family 835.

The monosomics are $a.g^3$, the triploids $a.g^3g^3$. In this cross more nullisomic gametes with the reduced chromosome number than with the unreduced have functioned. The result of crossing nulli- and disomics seems to be different when the nullisomic is the male parent. Perhaps restitution nuclei are formed more often in the ovules than in the anthers; certation is improbable, because the disomic mother had many ovules and the nullisomic is very pollen sterile.

Nullisomic. — A family 43—665 that had been produced through selfing a monosomic from a natural locality had two kinds of dwarfs; one had leaves of a dark-green colour, the other had leaves of a yellowish-green. Some plants were ordinary nullisomics, two were monosomics with 5 II + chain-of-three (HÅKANSSON, 1945). Now this family was sown again in 1944, and eight more plants could be investigated, four of the type with dark-green leaves, four of the type with yellowish-green leaves. The segregation was 29 normals (*a.a* and *a.g*):

47 dark-green dwarfs: 16 yellowish-green dwarfs (HIORTH, in a letter).

All plants investigated were nullisomics. Of several plants buds with meiosis stages had been fixed. In contradistinction to the I's of the tetraploid nullisomic all the I's here were not infrequently in an equatorial plate. The percentage of pollen diads was rather high. One plant had about 50 %, two had about 70 % diads. This is more than in the nullisomic plants first investigated (HÅKANSSON, 1943). However, plants from this family 665 fixed in 1943 also showed an unusual high percentage of diads. Thus, this family seems to have a tendency to form many pollen diads.

An aberrant monosomic × *disomic.* — The plant 1040/665—'43 had 5 II + chain-of-three. It was interpreted as $g.g$ + an a chromosome from the chain. A non-disjunction had occurred in the chain of the monosomic, an a chromosome having followed the g chromosomes. The presence of this a chromosome had changed the strong asyndesis of $g^3.g^3$ to about the degree that is found in $a.g^3$. Now, this plant had been pollinated with pollen from Bremen ($a.a$).

Two of the hybrid plants had 13, six had 14 chromosomes. Thus egg-cells with 7 chromosomes function more often than egg-cells with 6. On account of a certain elimination of a chromosome in the meiosis of 1040, this plant forms slightly more 6-chromosome gones than 7-chromosome gones. One would rather expect an excess of plants with 13 chromosomes in this cross; the reverse is, however, the case. One may compare the result when the ordinary monosomic $a.g$ is pollinated by a disomic. Then plants with 13 chromosomes are in a great majority in the progeny.

No stages of meiosis were found in plants with 13 chromosomes; the expectation here was normal $a.g$ pairing. Meiosis could be studied in four plants with 14 chromosomes. The typical pairing was rather difficult to ascertain, owing to the usual weak asyndesis. Fig. 2 *f* shows 4 II + I + chain-of-five. No doubt this is the typical configuration. Other configurations unmarred by asyndesis in bivalents were: 4 II + + 2 I + chain-of-four and 5 II + I + chain-of-three. All p.m.c's had at least one I. This I must be chromosome 5.6 that is present in the standard genomes of Bremen, but is assumed to be lacking in 1040. If the assumption that 1040 is $g^3.g^3$ and also has an a chromosome from the chain-of-four is correct, then the configurations now described would be expected to occur in plants with 14 chromosomes. This assumption may thus be considered as verified. But at the same time

it is proved that the asyndesis in the nullisomic is not a result of chromosome 5.6 being absent, but is due to the constitution $g^3.g^3$.

The *a* chromosome present in 1040 could be 1.2 or 3.4. If it is 3.4, 1040 should show the chain or III 3.4 — 4.3 — 3.2 and the II 4'.1 — 1.4' or 3.4 — 4.3 + 4'.1 — 1.4 + 3.2 (6 II + I). 1040 × *a.a* could give a chain-of-five, 4'.1 — 1.2 — 2.3 — 3.4 — 4.3; or perhaps more often 4'.1 — 1.2 — 2.3 and the II 3.4 — 4.3. If the chromosome was 1.2, then 1040 could sometimes show the chain 4'.1 — 1.2 — 2.3 — 3.2 and the I 1.4'. However, a chain-of-4 + I was not observed in 1040. And in 1040 × *a.a* the chromosomes 1.4', 1.2, 1.2, 2.3 and 3.4 could not form a simple chain-of-five (only a branched chain-of-five with a triple chiasma). But such a simple chain was observed. Thus it seems that the plant 1040 was $g^3.g^3$ and also had the *a* chromosome 3.4.

HORSE MTN.

In the progeny of monosomics $a.g^2$ there appears in a low percentage a very sterile type (the pollen sterility is about 99 %, HIORTH has communicated in a letter). This type has smaller flowers than normal plants and has a green stem, whereas the monosomic $a.g^2$ had a red stem. The green-stem type had 13 chromosomes and at metaphase 1 the pairing was always 13 I. At first this type, which shows certain nullisomic properties, was thought to be the result of a misdivision of the centromere of the univalent in $a.g^2$, but later on it was considered more likely that this form was $g^2.(g^2 + 1)$, a *g* gamete with 6 chromosomes having fused with a *g* gamete with 7 chromosomes.

Notwithstanding this great sterility some hybrids have been produced by HIORTH by crossing the type having a green stem with other Godetias. Some plants that had been produced by pollinating the green-stem type with pollen from C^a (*a.a*) have been studied. Buds with meiosis from four plants had been fixed. Two plants (44—804 c and d) were monosomics and had the pairing 4 II + I + chain-of-four, one plant (a) was a disomic with 5 II + chain-of-four, one plant (b) was a trisomic with 5 II + chain-of-five or 6 II + chain-of-three. A weak asyndesis occurred.

No unreduced egg-cells had functioned here. As 80 % regular and irregular diads are formed in the anthers, this is rather surprising. If meiosis in the ovules was similar to meiosis in the anthers, most (80 %) of the egg-cells should have 10—13 chromosomes. But no such egg-cell had functioned.

HYBRID MONOSOMICS AND HYBRID NULLISOMICS.

The hybrids had two different *g* complexes, or a *d* and a *g* complex.

Occidental $zw_1zw_1 \times$ *S. Rosa 6 monosomic.* — In *G. Whitneyi* there is a complex *d*, that with the standard complex *a* gives 5 II + I + + chain-of-three. This pairing is very constant. Plants with this chromosome configuration are known from several localities (HÅKANSSON, 1942). The cytogenetics of two *a.d* have been studied (HÅKANSSON, 1944; HIORTH, MS.), they are $a.d^1$ from Occidental and $a.d^2$ from Tocaloma. The former segregates a dwarf type with very strong asyndesis that is called zw_1 zw_1 and is the homozygous combination $d^1.d^1$.

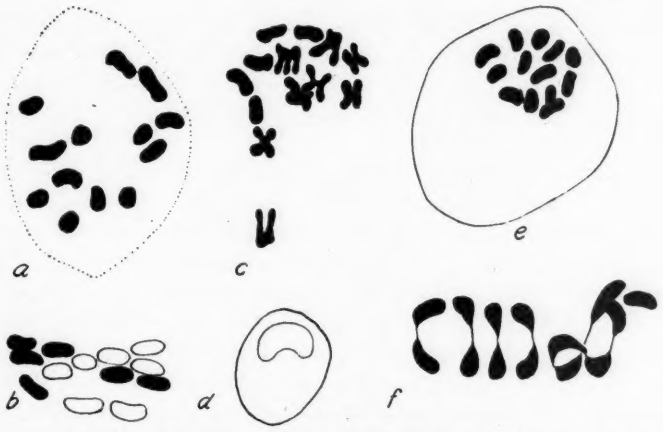

Fig. 2. *a*—*e*: Occidental $zw_1zw_1 \times$ monosomic S. Rosa 6. *a*: 13 I. — *b*: 13 I, the univalents here show a tendency to form a plate. — *c*: telophase 1. — *d*: p.m.c. with a restitution nucleus. — *e*: metaphase 2. — *f*: the combination $a.(g^3 + 3.4)$, 4 II + + I + chain-of-five.

The most common pairing in the dwarf is 1 II + 12 II. In $zw_1zw_1 \times$ Bremen the pairing shows that the dwarf forms d^1 gametes. The dwarf had been pollinated with pollen from the monosomic of S. Rosa. Some hybrid plants had been fixed, two of them had 13 chromosomes and thus must have the constitution $d^1.g^3$.

These plants showed a very strong asyndesis, all p.m.c's had 13 I, a II could never be observed. The position of the I in the p.m.c. is varying (Figs. 2 *a* and 2 *b*). There is a certain tendency to form a plate (Fig. 2 *b*), though a very weak one that only manifests itself in a minority of the p.m.c's. At interkinesis and at metaphase 2 most p.m.c's have a large nucleus or a group of many chromosomes and one

or more small nuclei or isolated chromosomes or small groups of chromosomes. The small nuclei lie near the wall of the cell but so does the large nucleus as well. Some chromosome distributions may be mentioned: 13, 12 + 1, 11 + 1 + 1, 11 + 2, 10 + 1 + 1 + 1, 8 + 2 + + 1 + 1, 8 + 1 + 1 + 1 + 1 + 1, and so on. The distribution of the I and the excentric position of the nuclei seem to have been brought about in the same manner as in the tetraploid nullisomic. Here, however, most of the I's are much more often included in one large interkinesis nucleus. Even if there is only one restitution nucleus containing 13 chromosomes, the position of this nucleus is excentric (compare Fig. 2 *d* and *e*).

After meiosis the loculi contain a very high percentage of regular diads or irregular diads with two large and one or more very small pollen-cells. A count in some anthers showed 93 diads of these two types, 20 polyads (with in most cases a high number of pollen-cells), and only 8 tetrads.

There is a striking resemblance between meiosis in $d^1.g^3$ and in $g^2.(g^2 + 1)$. Both combinations show a complete asyndesis with 13 I, close investigation never revealing a bivalent. Not only the degree of asyndesis but also the result of meiosis is very similar. About three-fourths or four-fifths of the pollen sporads are diads or irregular diads with two large cells and one or more dwarf cells.

Briceland—C^{st} × Horse Mtn. monosomic. — This cross was $a.g^1 \times$ $\times\ a.g^2$. Six hybrid plants could be investigated. They had deformed flowers, but the somatic chromosome number could always be determined in mitosis in young petals. Although none of the monosomics in this cross segregates a nullisomic, plants with 12 chromosomes appeared in the F_1. They must have the constitution $g^1.g^2$, and in two of them meiosis could be studied.

The nullisomics show a strong asyndesis. The pairing could be studied in 66 p.m.c's. This comparatively high number was a result of the rather large anthers of one of the plants investigated. 40 p.m.c's had 12 I (Fig. 3 *c*), while 26 had one or more II's. In most cases the number of bivalents was one or two, but as many as five could be counted (Fig. 3 *b*). Thus the pairing is better than in the nullisomic from Santa Rosa 6. Moreover, a heteromorphic II was observed. The chromosome configurations at metaphase 1 are distinctively different from those in $g^3.g^3$, and the heteromorphic II proves structural differences between g^1 and g^2.

Later stages of meiosis were lacking but some anthers with pollen

sporads were found. 40 polyads were counted and only 3 tetrads, but no diad. The polyads as a rule contained many pollen-cells. Thus one may conclude that there had been a great dispersion of the univalents at interkinesis, and an interkinesis nucleus containing many chromosomes must be a rarity in this asyndetic form.

Briceland — C^{st} × Tocaloma — C^{pa}. — This is also a cross between two monosomics neither of which segregates a nullisomic. Of the few investigated hybrids, however, three plants had 12 chromosomes. They have the constitution $g^1.g^4$. These plants had apparently a quite normal habitus, the anthers were, however, very small. This deformation of the flowers is not a result of an aberrant chromosome number or constitution. Monosomic sister plants also had deformed flowers.

Only in one of the nullisomic plants could some small loculi containing p.m.c's with meiotic stages be found. Their number was small. The pairing seemed to be still better than in $g^1.g^2$, no p.m.c. lacking univalents was observed, however. The lowest number of univalents observed was 2, the highest 10. Some of the pairings are shown in Fig. 3 *d—f*. In this nullisomic a chain of four chromosomes could be observed. At interkinesis all p.m.c's had more than two nuclei. As a matter of fact, the dispersal of the chromosomes was often very pronounced (Fig. 3 *g*). The second division had a very irregular appearance owing to the many spindles.

105 polyads, 14 tetrads and 1 or 2 diads were observed. Most polyads had a great number of pollen-cells. It is remarkable that after a relative good chromosome pairing (perhaps four is the most common number of II) such great irregularities in the distribution of the chromosomes occur. It is a result of a strong tendency to spread out the chromosomes before interkinesis sets in. The high frequency of polyads with in most cases many pollen-cells and the rareness of diads are conspicuous features of $g^1.g^2$ and of $g^1.g^4$.

The chromosome chains observed show structural differences of g^1 and g^4. Now, the genetical work of HIORTH has already shown that the ring or chain of Briceland — C^{st} cannot be composed of just the same chromosomes as the chain of Tocaloma — C^{pa}. The chain of the latter monosomic contains the chromosome that carries the genes *f* and w_1, this chromosome is not a member of the ring in the monosomic from Briceland. The small number of p.m.c's has not permitted a closer study of the chromosome chain that is sometimes formed in this nullisomic.

Lastly, it may be mentioned that this nullisomic has the normal habitus of *Godetia Whitneyi*; it is not a dwarf type.

Santa Rosa 6 monosomic × *Tocaloma* — C^{pa}. — This cross is $a.g^3 \times a.g^4$. Four plants were investigated, three were nullisomics ($g^3.g^4$). The flowers were normal and meiosis could be studied closely

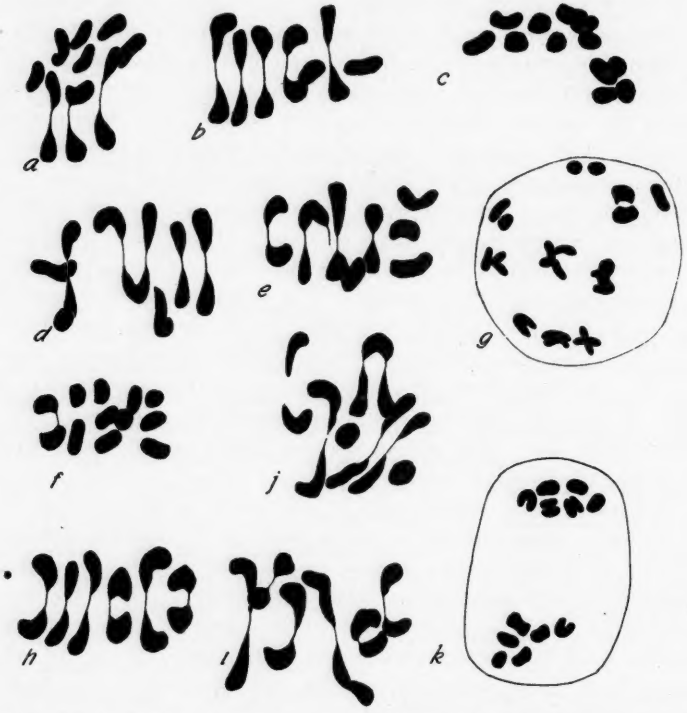

Fig. 3. Hybrid nullisomics. *a*—*c*: the complex combination $g^1.g^2$. *d*—*g*: the complex combination $g^1.g^4$. *h*—*k*: the complex combination $g^3.g^4$. — *a*: 3 II + 6 I. — *b*: 5 II + 2 I. — *c*: 12 I. — *d*: 3 II + 2 I + chain-of-four. — *e*: 2 II + 4 I + chain-of-four. — *f*: 1 II + 10 I. — *g*: telophase I. — *h*, *i*: 6 II. — *j*: the chromosomes of an aberrant p.m.c. with 13 chromosomes 1 III + 4 II + 2 I. — *k*: anaphase 1.

in the plant 645 c; there were also meiosis stages in another plant. This is the most interesting of the nullisomics.

It cannot be doubted that the normal configuration of this nullisomic is 6 II. In the plant c 73 p.m.c's had 6 II, while 46 had a lower number. Thus, most of the p.m.c's have 6 II. Chromosome chains were never observed, and no heteromorphy could be detected in any

of the bivalents. Hence structural differences could not be found between these two *g* complexes.

The 46 p.m.c's showing asyndesis had the following configurations: 32 had 5 II + 2 I, eleven had 4 II + 4 I, one had 3 II + 6 I, one had 2 II + 8 I, and one had 1 II + 10 I. This asyndesis is understandable, as $a.g^3$ shows a certain amount of it. There was often instability in the position of the bivalents at the equatorial plate (Fig. 3 *i*). The II formed by the larger end-chromosomes from the chains of the monosomics was sometimes conspicuous (Fig. 3 *i* the 4th II). Fig. 3 *j* shows the chromosomes from a p.m.c. with 13 chromosomes. The cell had a trivalent and there must have occurred a non-disjunction at the last premeiotic division. Such a cell was observed only once.

Anaphase 1 is as a rule regular, 6 chromsomes passing to each pole (Fig. 3 *k*). When univalents are present, lagging and elimination may occur. Rarely was a univalent divided. The result of the meiotic divisions is entirely different from that in other nullisomics of *Godetia Whitneyi*. Of 364 sporads there were 270 tetrads, 92 polyads (always with a moderate number of pollen-cells) and 2 diads. This high number of tetrads (about 75 %) is a result of the good pairing at metaphase 1, and a lack of such factors as caused the spreading of the chromosomes in $g^1.g^4$. The pollen fertility must be rather high.

Concerning these plants, Dr. HIORTH has communicated that he has no annotations on their morphology. Respecting a sister family, however, there is a note: C^{pa} plants (i. e. nullisomics) early, not so high as the sister plants, more slender branches. As Dr. HIORTH remarks, the plants probably had a quite normal appearance. Regarding their fertility, Dr. HIORTH writes that all four plants of the family 645 had given a progeny of more than 70 plants per isolated branch, the plant c (studied here) many more than 100. The fertility of these plants is thus relatively good; this is a fertile nullisomic. A *Godetia Whitneyi* has been produced with a new basic number previously not found in the genus *Godetia*, with a rather regular meiosis and a relatively good fertility. This *Godetia* is a heterozygous combination, $g^3.g^4$, and probably it should segregate the homozygous combinations $g^3.g^3$ and $g^4.g^4$. The former is as we know dwarfish, asyndetic and sterile. The latter is not viable, being a lethal zygote. Thus, in a certain sense the new combination may be said to be constant.

CONCLUSIONS.

Several genotypes of *Godetia Whitneyi* show a pronounced asyndesis or asynapsis. The degree of asyndesis is somewhat different in different genotypes, and the observations may be summarized thus: (1) Complete asyndesis, a bivalent was never observed, this was found in two types with 13 chromosomes, $d^1.g^3$ and $g^2.(g^2+1)$. (2) Very strong asyndesis, occasionally one or very rarely two bivalents are formed, this was found in di- and tetra-ploid nullisomics from Santa Rosa 6. (3) Many p.m.c's have one or two bivalents, some may show a higher number, this being observed in the dwarf $d^1.d^1$ and in $g^1.g^2$. (4) A higher number of bivalents are formed, probably three or four was the most common number in $g^1.g^4$.

In the asyndetic forms the position of the chromosomes in the p.m.c. at telophase 1 and interkinesis is often only in a minor degree a result of anaphasic movements through attraction fibres. Changes in the spindle and cytoplasm at stages corresponding to late anaphase 1 and early telophase 1 play an important part, isolated univalents or small groups of univalents are spread about but the changes are also able to move larger groups of chromosomes. Interkinesis therefore presents a variable picture, but there are also differences between different asyndetic forms. The chromosomes divide at anaphase 2 and the composition of the pollen sporads is conditioned by the position and size of the interkinesis nuclei. (1) At interkinesis there is in most p.m.c's one large nucleus containing all or most of the chromosomes, more than 50 % of the sporads are regular diads with two large pollen-cells or irregular diads with two large cells and one or more dwarf cells. This was found in $d^1.g^3$, in $g^2.(g^2+1)$ and in nullisomics belonging to a certain family of Santa Rosa 6. (2) Diads are frequent but they occur in a frequency of less than 50 %, in the first investigated nullisomic the frequency was 28 %. (3) At interkinesis there is a strong dispersion of chromosomes; this results in a high frequency of polyads, in most cases with a high number of small pollen-cells. The percentage of tetrads is low, diads are few or lacking. This was observed in $g^1.g^2$ and $g^1.g^4$. This result of meiosis in $g^1.g^4$ was rather unexpected, as the chromosome pairing was relatively good. Perhaps the separation of paired homologues was slow, with the result that the changes of the spindle and the cytoplasm set in before the chromosomes have assembled at their poles. The tetraploid nullisomic seems to have a meiosis that is intermediate between (2) and (3).

The result of crosses with the asyndetic forms is sometimes un-

expected. The nullisomic from Santa Rosa 6 may have a few functional egg-cells and pollen-grains. The egg-cells were in all cases but one unreduced, the pollen-grains had, however, 6 more often than 12 chromosomes; they were *g* or *gg*. The combination $g^2.(g^2 + 1)$ that forms such a high number of regular or irregular pollen diads was different, all functioning egg-cells were reduced, they had 6—8 chromosomes. The asyndetic dwarf $d^1.d^1$ seems to form egg-cells of one kind only, d^1 (HÅKANSSON, 1944; HIORTH, 1945). Here the pairing was better than in the former cases, and it seemed as though homologues paired at meiotic prophase often fell apart late. Thus, there is here a good chance that a complete *d* complex comes to lie on each side of the equator of the spindle.

Godetia Whitneyi has four different kinds of chromosome complexes. *a* or the standard complex is present in plants from all or most natural localities, it is also a member of most heterozygous complex combinations, *b* causes in heterozygous combinations rings very similar to the rings in *Oenothera*, *d* causes in *a.d* 5 II + I + ring-of-three, *g* has only six chromosomes, the combination *a.g* shows 4 II + I + chain-of-four. Homozygous combinations may show certain defects such as dwarf growth, a strong asyndesis or zygotic lethality. Some examples of defects have been given in this paper and in some earlier publications (HÅKANSSON, 1943, 1944, 1945). *G. Whitneyi* shows as a complex heterozygote greater variation than *Oenothera*. *Oenothera* has only *b* complex, *d* and *g* are not known and a standard complex does not occur. Homozygous combinations from *Oenothera* types with large rings are in most cases lethal, in a few cases they are dwarfs with 7 II, in some cases they have a normal habitus (RENNER, 1941), but asyndesis was unknown until recently. CATCHESIDE (1939) found a very strong asyndesis in the combination ^{h}*decipiens*. ^{h}*decipiens*, which segregates from the half mutant *erythrina* of *Oe. Lamarckiana*, a form with 4 II + ring-of-six. The cause of asyndesis was assumed to be a recessive gene in ^{h}*decipiens*.

The *g* complex very probably originated through unequal reciprocal translocation followed by a loss of that chromosome whose centromere after the translocations no longer carried any important segments. Such a process was assumed by DUBININ to explain the origin of a *Drosophila melanogaster* with n = 3 through X-raying. DARLINGTON (1937, p. 559) assumed such changes to be a mechanism of changes of the basic number of chromosomes. TOBGY (1943) studied the chromosome pairing in *Crepis fuliginosa* (n = 3) × *Cr. neglecta* (n = 4). He concluded that

the three *fuliginosa* chromosomes must have been produced by the four *neglecta* chromosomes in a manner that followed the scheme of DARLINGTON, and SHERMAN (1945; cited after STEBBINS, 1945) produced evidence that *Cr. Kotschyana* (n = 4) in a similar manner evolved from an ancestor related to *Cr. foetida* (n = 5). Thus, the mode of origin of the different *g* complexes in *G. Whitneyi* is easy to understand. The unequal translocation here was probably between a chromosome that was a member of a ring or a chain, i. e. a chromosome that had already been changed, and the chromosome 5.6. The new compound chromosome becomes a member of a configuration with a zigzag arrangement at metaphase 1, and the result is that the compound chromosome is preserved. It is clear that the defects appearing in the different *g* complexes, when they are realized as homozygous combinations, may be a consequence of secondary structural changes resulting from the unequal translocation. These may be of two kinds. First, certain segments of chromosome 5.6 are not incorporated in the *g* complex, the homozygotes are thus nullisomic for such segments. Second, segments of the ring-chromosome that changed with 5.6 may be lacking in the *g* complex.

Probably such deficiencies are the cause of the defects in the different *g* complexes. There is evidence that the strong asyndesis of $g^3.g^3$ is a result of a deficiency rather than of a recessive gene. Monosomics from Santa Rosa show a weak asyndesis. Thus *a.a* has no asyndesis, $a.g^3$ a weak, $g^3.g^3$ a very strong asyndesis. Now, the asyndesis in triploid $a.g^3g^3$ does not seem to be more pronounced than that of $a.g^3$. This speaks in favour of a loss of segment. If asyndesis was due to a recessive gene in the *g* complex, a stronger asyndesis would be expected in the triploid than in the monosomic. Many cases are known of a heterozygote of the type *Aaa* being more similar to *aa* than *Aa* is to *aa*; such a difference between heterozygotes would hardly be expected in a case of loss of segment. As is shown on page 501, the presence of one or two 5.6 chromosomes does not influence the pairing, but the presence of the *a* chromosome designated 3.4 changes the strong asyndesis of $g^3.g^3$ to a weak asyndesis. The missing segment in the g^3 complex thus has an equivalent in chromosome 3.4, and the asyndesis is a result of a loss in the changed chromosome of the ring when it made the unequal reciprocal translocation with 5.6, not to loss of a segment of 5.6.

It is probable that the zygotic lethal »factors» that are in three of the *g* complexes are also deficiencies or other structural changes. The

properties of the complex g^1 no doubt indicate that this complex is structurally different from other g's: the monosomic from Briceland often has a ring, with g^2 and g^4 the g^1 complex may form chains or heteromorphic bivalents, the chromosome carrying the genes f and w_1 is not a member of the configuration-of-four in the monosomic. But the zygotic lethal »factor» also must be different in g^1. This is evident from the fact that plants with 12 chromosomes appear in the crosses between the monosomic from Briceland and monosomics from Horse Mtn. or Tocaloma. Nullisomics thus appear in crosses between monosomics neither of which segregates a nullisomic. The hybrid nullisomics were $g^1.g^2$ and $g^1.g^4$. That these combinations not are lethal must depend on their not being homozygous for any lethal »factor». Thus, the lethal in g^1 must be different from the lethals in g^2 or g^4 and the influences of different lethals are not additive. As the complex g^1 has originated through other reciprocal translocations than g^2 and g^4 and as it has other lethal »factors» than g^2 and g^4, it seems probable that these factors are loss of segments occurring at the reciprocal translocations. The appearance of hybrid nullisomics in crosses Tocaloma —C^{pa} × monosomic Santa Rosa 6 is not surprising, as the g^3 complex has no lethal »factor».

In the hybrid nullisomics the asyndesis is not so strong as in homozygous g combinations. This may be expected if the deficiencies causing asyndesis are different in different g complexes. Perhaps certain g complexes lack such deficiencies; certain homozygous combinations would perhaps, if they could be realized, show normal pairing. The hybrid nullisomics from Tocaloma — C^{pa} × S. Rosa 6 had 6 II in most p.m.c's, meiosis was regular in most p.m.c's and 75 % of the pollen diads were regular tetrads. This hybrid nullisomic is fertile.

As was mentioned before, the homozygous g combinations are very striking types, morphologically different from normals and monosomics. In contradistinction the hybrid nullisomics show a normal habitus. Some hybrid combinations have more or less deformed flowers, but this has no relation to the problems discussed here; their monosomic sisters also had deformed flowers. Thus, the different g complexes compensate each other, hybrid combinations have a better chance of not being lethal, they have a more normal habitus and a better chromosome pairing than homozygous combinations.

The crosses made by HIORTH between different monosomics have no doubt given some results of rather general interest. The combination $g^3.g^4$ is a *G. Whitneyi* with the new chromosome number 6, pre-

viously unknown in the genus *Godetia*, with a regular meiosis, a high percentage of normal pollen tetrads and a good fertility. Perhaps this form has already been produced in nature.

But also the hybrid nullisomics $g^1.g^2$ and $g^1.g^4$ are of some interest. As each of them is composed of two complexes that cannot be realized as homozygotes, they must show balanced lethality, and a situation very similar to that of *Oenothera Lamarckiana* is realized here. The meiosis is, however, rather disturbed in these hybrid nullisomics and probably they have a high sterility.

Homozygotic combinations *b.b* and *d.d* may also in certain cases show dwarf growth, strong asyndesis or zygotic lethality. *b* and *d* complexes have 7 chromosomes, different *b*'s and *d*'s must have originated through different reciprocal translocations. It is probable that asyndesis and other defects are a result of minor structural changes such as deficiencies, that changes may thus also follow such reciprocal translocations as do not lead to a change in chromosome number.

Addendum. — Hybrid nullisomics have also appeared in the cross Tocaloma — $C^{Pa} \times$ monosomic Horse Mtn. They must be the complex combination $g^2.g^4$. Chromosome pairing in these nullisomics was surprisingly good. In some p.m.c's 4 II + chain-of-four were observed. The chain was not similar to the chain in the monosomics crossed. In the monosomics the end-chromosomes of the chain were of different size; in the nullisomic the end-chromosomes had about equal size, and they were large chromosomes. It is clear that the latter chain was formed by the *g* chromosomes from the two monosomics, and thus the chain in the monosomic from Horse Mtn. is composed of other chromosomes than the chain in the monosomic from Tocaloma. Anaphase 1 was, however, very often disturbed in $g^2.g^4$, most p.m.c's showing several lagging chromosomes. Usually more than two interkinesis nuclei were formed.

SUMMARY.

Plants of *Godetia Whitneyi* with two or more *g* complexes (*g* complexes have 6 chromosomes) or a *d* and a *g* complex show a strong asyndesis. The degree of asyndesis is different in different combinations, the position of the chromosomes at interkinesis and the number of cells in the pollen sporads are also different in different asyndetic forms. Changes in the spindle and the cytoplasm in the p.m.c. at stages corresponding to late anaphase 1 and early telophase 1 determine the position of the chromosomes at interkinesis.

Through crossing different monosomics some hybrid nullisomics have been produced. They are composed of two different *g* complexes. Hybrid nullisomics have a better chance of being vital, a better chromosome pairing and a more normal habitus than such nullisomics as are homozygous realizations of *g* complexes.

Crossing two monosomics neither of which segregates a nullisomic may produce hybrid nullisomics. Such hybrid nullisomics must show a condition of balanced lethality similar to that of many Oenotheras.

A fertile nullisomic with 6 II and a regular meiosis was detected in one of the crosses. Here a *Godetia Whitneyi* with the basic number 6 had been produced.

There is evidence that those defects in the different *g* complexes which manifest themselves in the homozygous combinations are caused by loss of segment or other secondary structural changes; probably they originated at the same time as the unequal reciprocal translocations that have led to the forming of the complex with 6 chromosomes.

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CHROMOSOME NUMBERS IN ANEMONE NEMOROSA AND A. RANUNCULOIDES

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AS a link in a study of the polymorphy of *Anemone nemorosa* L. and *A. ranunculoides* L. and its causes the chromosome number has been determined on a rather large number of specimens collected in nature from both the species. Each specimen forms a clone. As the investigation will be a lengthy one, the determinations are submitted already now, since they have their value for comparison with other counts published by various authors for these species.

The counts were exclusively made on root-tips. The fixations were made in the winter of 1942—1943 during November—February at the growing places. As fixative two Craf variants were used, for the most part LANGLET's (LANGLET, 1932), to a less extent RANDOLPH's (RANDOLPH, 1935), both being found satisfactory. Gentian violet was used as stain. The roots were cut to 15 μ.

The chromosomes were not always easy to count. Their number is given as certain if two or more good plates, counted without difficulty, indicated the same number. $\pm$ in front of a number shows that most of the counts gave this number. When double numbers are given, each of these were found as often as the other, in spite of several counts being undertaken. Where one of the numbers is within brackets, this number was indicated by fewer or less certain counts than the number outside the brackets. It must however be emphasized that judging from the list given below the possible cases of aneuploidy may depend solely on miscounting. Only No. 29 seems really to be an aneuploid, maybe also No. 9.

In three numbers what are assumably accessory chromosomes were observed; more than one were never seen in the same plate. The chromosome in No. 69 was rather large, those in Nos. 24 and 117 were small. A 30-chromosomal *nemorosa* had $\pm$ 60 chromosomes in a broad sector of one root. One *ranunculoides* clone had distinctly smaller chromosomes than the others, although the plants were tall in growth. Somatic chromosome-number, doubling and varying chromosome sizes have been previously observed in *Anemone*.

The numbers in the list are those of the clones themselves in the cultures. All the chromosome numbers are the somatic ones.

Anemone nemorosa.

No.	2	45	Billingen, Västergötland
	3	± 30	» »
	4	30—31	» »
	6	45	» »
	7	45	» »
	8	30	Botanical Garden, Lund. Cult. (var. *alba plena*)
	9	± 43	Dalby, Skåne
	24	± 30 +	access. chrom. Torup, Skåne
	25	30	Torup, Skåne
	27	± 30	Kungsmarken, Skåne
	31	30	Botanical Garden, Lund
	33	30	» » »
	48	29—30	Ignaberga, Skåne
	50	30	Eskilstuna, Södermanland
	54	29—30	Säbrå, Ångermanland
	55	30	» »
	56	30	» »
	63	± 30	» »
	67	(29—)30	» »
	69	± 45 +	access. chrom. Dalby, Skåne
	71	30	Konga, Skåne
	73	29—30	Hässjö. Medelpad
	81	30	Tumbo, Södermanland
	84	30	Sundsjö, Jämtland
	91	30	Västerhejde, Gotland
	92	30	» »
	94	30	» »
	96	30	» »
	100	30	Bondkyrko, Uppland
	103	30	Eskilstuna, Södermanland
	108	30	Rödö, Jämtland
	109	30	» »
	110	30	» »
	112	45—46	Weibullsholm. Cult. (var. »Blue Beauty»?)
	113	37	» » (unknown var.)
	116	30	» » (var. »Bracteata plena»)
	117	45 +	access. chrom. Weibullsholm. Cult. (var. »Grandiflora»?)

Anemone ranunculoides.

No.	19	± 32	Botanical Garden, Lund. Cult. (var. *subintegra*)
	29	± 31	» » »
	30	(31—)32	» » »

40	32	Brönnestad, Skåne
41	32	Vinslöv, »
43	32	» »
46	32	Ignaberga, »
85	32	Västerhejde, Gotland
90	32—33	» »
107	31—32	Berg, Jämtland

Anemone nemorosa × *ranunculoides.*

No. 1	(30—)31	Kullavik, Halland	
18	31	Botanical Garden, Lund	
22	31	» »	»
28	31	» »	»
36	(30—)31	» »	»
38	31	» »	»

The examined specimens of *nemorosa* are derived from the larger part of the area over which the species is distributed in Sweden, from the most southern point to the middle of the country, at latitude 63. The 45-chromosomal forms were all found in the southern part of the country. The *ranunculoides* specimens were collected partly in southern Sweden, partly at the northernmost localities of the species in the country, viz. in Jämtland. Numbers with »Botanical Garden, Lund», denoted as their locality of origin, were collected there as wild-growing plants, and probably descend from earlier transplantations from the province.

For purposes of comparison a list follows of earlier reports on the chromosome numbers in *Anemone nemorosa* and *ranunculoides.* The numbers given are the somatic ones.

WINGE, 1917	*nemorosa*	24	Denmark	
BÖCHER, 1932	»	28—32	»	
	ranunculoides	32	»	
	nemor. × *ranunc.*	abt. 30	»	
LANGLET, 1932	*nemorosa*	30	»	and Sweden
	v. *monoica*	30		
	v. *cornubiensis*	30		
	v. *phyllantha*	30		
	ranunculoides	32	Sweden	
MOFFETT, 1932	*nemorosa*	32, 39	England	
GUINOCHET, 1935	»	16	France	

BÖCHER could not determine the chromosome number exactly; he studied it only at meiosis. MOFFETT's 39-chromosomal form is prob-

ably purely an incidental one, perhaps a product of culture, like the 37-chromosomal one from Weibullsholm counted by me.

The number $2n = 8$ has not been found in any species within the family Ranunculaceae. On the strength of the numbers then known within the Ranunculaceae LANGLET (1932) assumes however that the basic number in at least *Anemone* and *Ranunculus* is $n = 4$. On various cytological grounds BÖCHER (1945) also suspects that *Anemone apennina* descends from a species or hybrid with $2n = 8$. If the basic number in *Anemone* is 4, the Swedish *nemorosa* is a hypo-octoploid, which in its turn has given rise to hypo-duodecaploid forms (12-ploid — 3). If the 16-chromosomal *nemorosa* is not a tetraploid but a diploid, then the 24-chromosomal form found by WINGE is a triploid, which in that case probably arose through a cross between a 16-chromosomal and a 32-chromosomal race found respectively in France and England. Knowledge of the triploid's sexual power of reproduction would be of great interest, although it is of little importance for the actual existence of the race, the species having great possibilities of continuing to live by means of rhizomes.

It is of interest that among different *Anemone* species with the somatic numbers 16 and 32 MOFFETT has found two, *A. baldensis* and *decapetala*, with $2n = 24$. Concerning these, he only remarks that they »obviously cannot be original species». Respecting *Anemone montana* from the section *Pulsatilla*, to which *nemorosa* does not belong, GUINOCHET states that it has been found to embrace the somatic numbers 16, 24, 32 and 48. The likeness of these to the series of numbers found for *nemorosa* is great and accentuates the uncertainty of the significance of the chromosome numbers of this and *montana*.

GREGORY (1941) includes in his list of the chromosome numbers within Ranunculaceae about 50 species of *Anemone*, of about 90 existing ones. On the basis of the numbers in the genus and the whole family, as well as on the circumstance that $n = 4$ has not been found within the Ranunculaceae, he rejects the idea that 4 is the basic number of *Anemone* and puts this at 8. Disregarding that group of the genus which seems to have the basic number 7, and only counting those species which have multiples of 4, about 60 % of the examined species have $2n = 16$, about 30 % have $2n = 32$ (or 30), about 7 % have $2n = 24$, and no species has $2n = 48$ (and 45), only forms of two species that also have a lower chromosome number. The percentages calculated by me cannot be given exactly owing to the uncertain taxonomy. The figures set forth, however, suggest that the basic

number is 8 and not 4. If it had been 4, a somewhat more even distribution of the different multiples of 4 might have been expected and not so great an accumulation of $2n = 16$ and 32. The question of the basic number cannot be decided yet, however, but hereafter in this paper the forms with 30—32 chromosomes will be called tetraploids and those with 45, hexaploids, since these designations seem to be the most correct.

The hexaploids in *nemorosa* are morphologically very much like the tetraploids. They are mutually well separated, which is to be expected in so polymorphic a species as *Anemone nemorosa*. Four clones collected from nature have been studied; No. 69 died soon after its discovery. No. 9 is very tall, No. 7 is robust although not conspicuously so, Nos. 2 and 6 are almost quite like tetraploids. There is no macroscopic character by which the hexaploids can be definitely distinguished from the tetraploids; tetraploid forms which in one or more characters invade the sphere of variation of the hexaploids are always to be found. The best signs of the hexaploids are doubtless somewhat, though not always, larger and perhaps yellower anthers and at the same time somewhat broader, rounder and whiter petals than normally. The hexaploids cannot be recognized by any character of the leaves, but the thickness of their stem is a shade greater than that of the tetraploids. Hexaploids cannot with certainty be recognized by the size of their seed.

Preliminary measurements of the breadth of newly opened as well as old flowers in the hexaploids and several of the tetraploids show that the difference in flower-breadth between the two categories in the newly opened flowers is very small, while in old flowers it is greater, although neither of these differences is significant. Tetraploids with equally large flowers as the hexaploids or still larger are common.

There is no general difference in earliness of development or flowering between the tetraploids and hexaploids. In length of flowering period the latter do not appear to differ from the former, or do so very little.

The length of the stomata appears to be a sure sign for the hexaploids. However, too few numbers have been examined to allow of a definite statement. On the other hand, extensive measurements have been made of the pollen-size in the four hexaploids and in 16 different numbers of tetraploid *nemorosa*. The hexaploids possess a pollen that, statistically, is significantly larger than the tetraploids ($P < 0{,}001$); for

the former the means lay between 8,29—8,46, and for the latter between 6,60—8,17 (2,75 parts correspond to $^1/_{100}$ mm.).

The quality of the pollen has been studied three years in succession. It varies considerably as between different years, which is not surprising, since meiosis takes place late in the autumn. The percentage of good grains varies much between different numbers in the tetraploids, and the general impression given is that the variation within the material approximates that shown by MÜNTZING (1939) for *Anemone nemorosa*. The hexaploids have rather good pollen-fertility; Nos. 2 and 9 have between 50-> 80 % good grains, while Nos. 6 and 7 have 70-> 90 %. The seed-setting also varies considerably within the material studied. Difficulty is often experienced in determining its size, as some of the seeds are very badly set, although they develop well. Such badly set seeds do not occur so much in the tetraploids as in the hexaploids, in which latter they are in the majority. In Nos. 2, 6 and 7 (No. 9 had too few flowers) the seed-setting percentage in 1943 was 60—80, but the percentage of good seeds was only 10—30. In No. 9 it seems to be < 10. In these hexaploid forms the figures indicating the number of seeds set probably show the percentage of functional gametes far better than does the percentage of good pollen-grains.

The odd chromosome number in the hexaploids and the poor seed production with a great frequency of half-set seeds make it clear that they have arisen through the functioning of an unreduced and a reduced gamete and that they cannot reproduce themselves sexually with retention of the chromosome number. Experiments have shown that the seeds cannot develop through uninduced apomixis. Altogether 15 flowers from three of the hexaploid numbers were emasculated and isolated. No seed was formed. Altogether 50 flowers in eight different numbers of tetraploid *nemorosa* were treated in the same way; none of them produced any seed.

With reference to *A. ranunculoides* it may be pointed out that, although several very tall or in other ways outstanding plants were examined, none with another chromosome number than that normal for the species were obtained. Like *nemorosa*, the species is very polymorphous. Unexpectedly enough it has been found to have on the average much better pollen-fertility than *nemorosa*.

From the chromosome numbers of the hybrids it is evident that *ranunculoides* crosses with the 30-chromosomal *nemorosa*. All hybrid clones studied by me are very ♀-sterile, setting only 0 — a few ripe

seeds per floret. BÖCHER (1932) describes similar conditions in Danish hybrid forms. On the basis of the pollen from dried material he has however discerned two types of hybrids, distinguished by the percentage of good grains. It is conceivable that one of the hybrid types originates from the 30-chromosomal *nemorosa*, the other from the 32-chromosomal.

Crossing and progeny experiments of various kinds are being carried out, but on account of the slow development of the germinal plants it will be some time before the results can be reported.

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STERILITY IN RYE POPULATIONS

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THE following data were gathered in the years 1937—39. In 1937 pollen fertility was studied in a number of self- and cross-fertilizing plant species. As previously reported (MÜNTZING, 1939, Table 1), a quite clear average difference was observed between these two categories. In six autogamous species the pollen was of a very good quality and of uniform appearance. Only in two of these species were a few plants found to have less than 90 per cent good pollen. In 10 different allogamous species, on the other hand, pollen fertility was much more variable, the percentages of partially sterile plants (with more than 10 per cent bad pollen) ranging from 10,0 to 85,4. One single allogamous species, *Cannabis sativa*, was exceptional, the percentage of apparently good pollen grains being as high as in the autogamous species.

The material of cross-fertilizing species also included rye, *Secale cereale*. Altogether 610 plants had been examined (l. c., p. 336) and of these no fewer than 50,2 per cent were partially sterile, the percentages of good pollen in these plants ranging from 0 to 90.

The high frequency of partially pollen sterile plants in ordinary populations of rye was surprising and led to a more detailed study. During this work the degree of seed setting was also observed and the inheritance of male as well as female sterility studied by examination of progenies from open-pollinated mother plants.

I. THE OCCURRENCE OF PARTIAL STERILITY IN DIFFERENT RYE POPULATIONS.

1. MALE STERILITY.

The material available chiefly consisted of the well-known commercial variety »Stålråg» (Steel-rye) and of a more primitive local variety »Östgöta gråråg». In 1937 the material of the former variety comprised 9 different short rows, sown as standards in a field with inbred rye, and one long row, used as a standard in an X-ray experiment. The long row had been sown later in the preceding autumn

than the short rows and was less well developed. On an average the plants were lower in height and also later in flowering than the plants in the short rows. These vegetative differences were also correlated with a difference in the average pollen fertility, the following values being obtained.

	Per cent good pollen											
	0—10	— 20	—30	—40	— 50	— 60	— 70	— 80	—90	— 100	n	$\bar{x}$
The long row:	1	—	3	4	6	14	8	25	30	28	119	74,50
The short rows:		2	—	—	—	—	1	6	31	43	83	87,53

The values in the table represent the average percentages of apparently good pollen per plant, 119 individuals in the long row and 83 individuals in the short rows having been examined. Evidently pollen fertility was better and less variable in the short rows than in the long row. This difference is significant. A χ^2 test, using the classes 0—70—80—90—100, gives a χ^2 of 37,56 and a P much smaller than 0,001. On an average the two categories may be considered isogenous, the same seed sample of the same commercial variety being used in both cases. Thus, the conclusion must be drawn that the observed difference in pollen fertility is entirely caused by environmental differences. The correlation between vegetative development and fertility will be considered in detail below (p. 535). At present it is sufficient to realize that the degree of pollen fertility in population plants of rye is rather sensitive to environmental conditions.

Under such circumstances the question may be raised whether the partial pollen sterility in rye might not be caused entirely by environmental fluctuations, no genotypical differences between the plants being involved. This question was tested by taking several pollen samples of the same plant at different intervals and comparing the intra- and inter-plant variation. The samples were taken on 13 different days during the period 11th of June—2nd of July, 1937. As samples were only taken from flowering heads, the same plant could not be examined on all of the 13 days, and in many cases only one sample per plant was gathered. More precisely 40 plants in the »short rows» were only examined once, the remaining 42 plants being examined from 2 to 5 times. The corresponding figures for the »long row» were 73 plants only examined once and 46 plants examined from 2 to 8 times. In most cases the different samples of the same plant were taken on different days, in a few cases more than one sample was taken on the same day but from different heads.

Analyses of variance were made in the two categories separately. In the »short rows» a working mean of 85,0 was used, in the »long row» the corresponding value was 75,0. The final results were the following:

	The short rows			*The long row*		
	Degrees of freedom	Sums of square	Mean square	Degrees of freedom	Sums of square	Mean square
Between series:	81	353,16	4,3600	118	644,52	5,4620
Within » :	80	61,09	0,7636	87	280,13	3,2199
Total:	161	414,25		205	924,65	

For the short rows the v^2 value will be 5,710, giving a P smaller than 0,001. For the long row v^2 will be 1,696 and P intermediate between 0,01 and 0,001. This P value, however, is very close to 0,001, the v^2 exactly corresponding to $P = 0{,}001$ being 1,723.

The conclusion to be drawn from these calculations is evidently that there are significant plant differences with regard to pollen fertility. The percentage of good pollen may be rather variable in the same plant but nevertheless the inter-plant variation is stronger than the intra-plant variation.

The primary material in this case is too large to be tabulated in its entirety, but in order to illustrate the type of variation observed the values from 38 plants of the long row, in which more than one pollen sample per plant was taken, are given in Table 1.

Further data on pollen fertility in the same variety, »Stålråg», were gathered in the following summer, 1938. Pollen samples were taken from two different standard plots »10—51» and »3—59» in a field with inbred rye. Of »10—51» 35 plants were examined, of »3—59» 84 plants. From each plant two pollen samples, *a* and *b*, were taken from different heads. The percentage of good pollen was found to vary as follows:

	Percentage of good pollen							
	50 — 60	— 70	— 80	— 90	— 100	n	$\bar{x}$
»10—51», *a*		2	6	15	12	35	85,57
» , *b*		2	4	20	9	35	85,29
»3—59», *a*	2	1	8	28	45	84	88,45
» , *b*	2	2	8	29	43	84	87,98
Total	4	7	26	92	109	238	87,39

The total average, 87,39, is almost exactly the same value as the average pollen fertility in the »short rows» (87,53) studied in the preced-

TABLE 1. *Inter- and intra-plant variation in pollen fertility, »Stålråg» 1937.*

Plant number	Per cent good pollen										Number of samples
	0 — 10	— 20	— 30	— 40	— 50	— 60	— 70	— 80	— 90	—100	
15— 1									1	1	2
— 2						1	—	2	2		5
— 4				1	—	1					2
— 6										4	4
— 8										2	2
— 9			1	—	1	—	3				5
— 10			1	—	—	—	—	—	1	2	4
— 11									1	1	2
— 14									2	2	4
— 15									2	1	3
— 16									1	1	2
— 18									1	1	2
— 20			2	—	—	—	—	—	—	4	6
— 27		1	—	—	1						2
— 29							1	—	1		2
— 37										2	2
— 43									1	1	2
— 46					2	1					3
— 47								1	—	1	2
— 49							1	1			2
— 53										2	2
— 55										2	2
— 62									2		2
— 64						1	—	3			4
— 65							2	—	1		3
— 67			1	—	—	—	1	1			3
— 72							2	—	1		3
— 75									1	1	2
— 76					1	1	—	—	—	2	4
— 77									2	1	3
— 81					1	2	1	1	1	2	8
— 84										3	3
— 85						1	—	—	1		2
— 88					1	—	—	1	1		3
— 92								1	—	2	3
— 94									1	1	2
— 95						1	—	—	4		5
—103			1	1	1						3

ing year, and again plants with less than 90 per cent good pollen are numerous. There is a slight difference between the values of the two plots, the average value being higher in »3—59» than in »10—51». Moreover, the maximum class in the »10—51» series is situated between 80 and 90, whereas in the »3—59» series it is situated between 90 and 100. A χ^2 test, using the classes 50—80—90—100 and the sample values as variates, showed these differences to be rather significant, χ^2 being 9,99 and P intermediate between 0,01 and 0,001. According to the records, all the samples of »10—51» and one-fourth of the samples of »3—59» were taken on the 14th of June, the majority of the samples of »3—59» being collected two days later. Slightly different environmental conditions on the two days of collection may be responsible for the slight difference in pollen fertility observed.

In both groups of material the correlation between the percentage values of the *a* and *b* samples of the same plant was studied. In the 35 plants of »10—51» the coefficient of correlation was found to be $+ 0{,}5541$ ($t^2 = 14{,}619$ and $P < 0{,}001$), in the 84 plants of »3—59» the same coefficient was found to be $+ 0{,}5561$ and P again smaller than 0,001. Thus, in this case again, the intra-plant variation was smaller than the inter-plant variation.

In the same year pollen fertility in »Stålråg» was compared with pollen fertility in another rye variety, »Östgöta gråråg». Pollen samples were taken from neighbouring plots in a comparative field trial. On account of dense (machine) sowing separate plants could not be distinguished. However, the pollen samples were taken from single heads at some distance from each other and thus certainly representing different individuals. 119 samples were taken from each variety, the following values being obtained:

	Per cent good pollen								Per cent partially
	50—60	60—70	70—80	80—90	90—100		n	$\bar{x}$	sterile plants
»Östgöta gråråg»:	2	5	14	38	60		119	87,52	$49{,}58 \pm 4{,}58$
»Stålråg»:			7	31	81		119	91,22	$31{,}93 \pm 4{,}27$

Though slight, the difference in distribution in the two series is significant. A χ^2 test with the classes 50—80—90—100 gave a χ^2 of 10,84 and a P intermediate between 0,01 and 0,001. The frequency of partially sterile plants with less than 90 per cent good pollen is higher in »Östgöta gråråg» than in »Stålråg». The difference between

the percentage values is $17{,}_{65} \pm 6{,}_{25}$, giving a t of $2{,}_{82}$ and a P intermediate between $0{,}_{01}$ and $0{,}_{001}$.

The pollen samples were taken during a period of 7 days and, thus, it is not quite excluded that the difference may be due to changes in the environment during these days. It is more probable, however, that the cause is an average genotypical difference between the two varieties. This supposition is strengthened by a significant difference between the two varieties in the amount of seed setting of the same plants as were used for the pollen examinations (cf. below p. 527).

Finally, it remains to mention that partial pollen sterility was not only observed in »Stålråg» and »Östgöta gråråg» but also in two other rye varieties. In »Kungsråg» 7 plants of 11 were partially sterile and in the variety »0305» 6 plants of 9 had less than 90 per cent good pollen. Though fragmentary these data strongly indicate that the occurrence of partially pollen-sterile plants is a rather universal phenomenon in rye populations.

2. FEMALE STERILITY.

In the material of »Stålråg» and »Östgöta gråråg» described above the percentage of seed setting in open-pollinated heads was also determined. The results obtained are summarized in Table 2.

Considering first the values of 1937 it is immediately seen that the plants in the »long row» have a much lower percentage of seed setting than the plants in the »short rows». The causes of this significant difference may in part be the same as those responsible for the difference in pollen fertility discussed above. Another probable cause is the later flowering time of the plants in the long row and hence a sparse and

TABLE 2. *Percentage of seed setting in open-pollinated plants.*

Year and variety	0	10	20	30	40	50	60	70	80	90	100 %	n	$\bar{x}$
1937, Stålråg, »the long row»	7	7	10	10	23	14	17	7	3			98	$45{,}_{20}$
1937, Stålråg, »the short rows»	1	1	—	1	2	9	6	23	20	12		75	$74{,}_{60}$
1938, Stålråg, 3—59					2	2	4	19	46	11		84	$81{,}_{43}$
» , » , 10—51						3	1	10	18	3		35	$79{,}_{86}$
» , » , field trial						5	8	26	51	23		113	$81{,}_{99}$
» , Östgöta gråråg, field trial		2	3	8	8	14	12	28	25	4		104	$66{,}_{25}$

partly insufficient degree of pollination. At any rate, the very marked difference is only due to the environmental conditions.

In 1938 seed setting in the population plots »3—59» and »10—51» of »Stålråg» was about the same (81,43 and 79,86 respectively), the slight difference not being significant. The large plot in the field trial of the same variety gave about the same value (81,99). The comparable plot of »Östgöta gråråg», on the contrary, had a much lower value (66,35), the amount of seed setting in this variety being highly variable and on an average rather low. The difference was tested by an analysis of variance, giving a v^2 of 57,63 and a P much smaller than 0,001. Thus, differences in seed setting in open-pollinated population plants of rye may either be caused by genotypical or environmental causes.

II. CORRELATION BETWEEN MALE AND FEMALE STERILITY.

In all the groups of material reported in Table 2 the correlation between degree of pollen fertility and degree of seed setting in the same plant was tested. The »long row» of »Stålråg» studied in 1937 showed a significant positive correlation, the value of r being $+ 0{,}3873$, t^2 16,76 and $P < 0{,}001$. Although the entire correlation table cannot be published, the following figures may illustrate the correlation in question:

Per cent good pollen:	20	40	50	60	70	80	90	100
Average percentage of seed setting:	23,0	35,0	37,3	41,7	43,7	56,3	47,2	
Number of plants:	5	4	13	6	23	24	23	

In the »short rows» of »Stålråg» examined at the same time as the »long row» there was also a positive correlation, but in this case it was not significant, r being as low as $+ 0{,}1215$, $t^2 = 1{,}097$ and $0{,}4 > P > 0{,}3$. The distribution of the variates was the following:

Per cent good pollen:	10	80	90	100
Average percentage of seed setting:	69,3	72,3	77,3	
Number of plants:	7	29	39	

In 1938 the field plot of »Östgöta gråråg» showed a significant positive correlation, r being $+ 0{,}4165$, t^2 21,20 and $P < 0{,}001$. The variates showed the following distribution:

Per cent good pollen:	50 — 70	— 80	— 90	— 100
Average percentage of seed setting:	43,3	55,0	65,3	72,3
Number of plants:	6	12	33	52

In the field plot of »Stålråg» observed at the same time the correlation was weaker, but still rather significant, r being $+ 0{,}2156$, t^2 4,877 and P intermediate between 0,05 and 0,02. The distribution of the variates in this case was as follows:

Per cent good pollen:	70 — 80	— 90	— 100
Average percentage of seed setting:	78,3	79,5	84,0
Number of plants:	6	29	67

A weak correlation was also observed in the two population plots of »Stålråg» »3—59» and »10—51», growing in another field. As the distributions in these two plots were rather similar, the variates may be combined to a single correlation table. The r value in this case was found to be $+ 0{,}2880$, t^2 10,58 and P just slightly greater than 0,001. The distribution of the variates was the following:

Per cent good pollen:	50 — 70	— 80	— 90	— 100
Average percentage of seed setting:	65,0	79,4	81,8	82,5
Number of plants:	5	16	62	36

The data so far given are from unselected plants taken direct in the populations. The correlation between pollen fertility and seed setting was also studied in progenies of the primary population plants. Thus, 86 progenies were raised from the open-pollinated plants in the »long row» of »Stålråg» studied in 1937. These progenies comprised a total of 617 plants. From the »short rows» of the same variety 70 progenies were raised, comprising a total of 521 plants. In both these groups of plants the correlation in question was examined. In »1938—253» (the progenies from the »long row») there was a slight but significant positive correlation, r having the value $+ 0{,}1576$, t^2 being 15,5380 and P smaller than 0,001. The distribution of the variates may be given as follows:

Percentage of good pollen:	30 — 60	— 70	— 80	— 90	— 100
Average percentage of seed setting:	71,4	74,5	81,3	84,2	81,5
Number of plants:	14	44	136	258	161

In »1938—254» (the progenies from the »short rows»), on the contrary, there was no trace of a positive correlation. A calculation of r gave the negative value $-0{,}1346$, which was found to be rather significant, t^2 being $9{,}5671$ and P intermediate between $0{,}01$ and $0{,}001$. The variates in this case showed the following distribution:

Percentage of good pollen:	30 — 70	80 — 90	90 — 100
Average percentage of seed setting:	82,1	79,3	78,7	76,8
Number of plants:	7	49	219	246

If the average seed setting and the average percentage of good pollen *per progeny* are used as variates instead of the values of the individual plants, the result was somewhat different. In this case a significant positive correlation was again found in »1938—253», r being as high as $+ 0{,}3654$ ($t^2 = 12{,}9417$ and $P < 0{,}001$). The distribution of the variates was now the following:

Per cent good pollen:	70 — 75	75 — 80	80 — 85	85 — 90	90 — 95
Average percentage of seed setting:	77,50	72,5	80,11	82,50	86,14
Number of progenies:	6	7	23	39	11

In »1938—254», on the contrary, the use of the average values of the progenies instead of the individual values resulted in an r value of $+ 0{,}0158$. Thus, the previous negative correlation had disappeared, the seed setting and pollen fertility in this case being completely uncorrelated.

Additional evidence was obtained from progenies of population plants of »Stålråg» and »Östgöta gråråg» studied in the following year, 1939. A total of 331 progenies were available from open-pollinated mother plants of »Stålråg» and 196 progenies of »Östgöta gråråg». In most cases the number of plants per progeny ranged from 20 to 30. The percentage of seed setting was determined in all these plants and, thus, the average percentage of seed setting per progeny is rather accurate. For practical reasons pollen samples could only be taken from 5 plants per progeny. Anyhow, as the total number of progenies was 527, this means that pollen fertility was examined in 2635 plants. The average per cent of good pollen per progeny (mean value of 5 plants) and the average per cent of seed setting per progeny (mean value of generally 20 to 30 plants) were correlated.

In both varieties tested a significant positive correlation was established. In »Stålråg» r had the value $+ 0{,}1506$, t^2 being $7{,}64$ and P

intermediate between 0,01 and 0,001. The variates showed the following distribution:

Per cent good pollen:	70 — 80	80 — 82,5	82,5 — 85,0	85,0 — 87,5	87,5 - 90,0	90,0 — 92,5
Average percentage of seed setting:	72,1	79,3	79,7	79,7	80,1	79,8
Number of plants:	12	18	38	74	118	71

The correlation is evidently quite weak and its significance only possible to demonstrate in a rather large material. Now this material is a total of different groups of progenies, viz. (a) 119 progenies of mother plants taken at random among the »Stålråg» plots »10—51» and »3—59», (b) 115 progenies from unselected mother plants of the field plot 1938—7 of the same variety, (c) 42 progenies from mother plants of the same plot having an especially good seed setting, and (d) 40 progenies from mother plants with an especially bad seed setting (less than 80 per cent). Originally groups (c) and (d) comprised 50 progenies each, but 18 progenies were not used for these separate calculations. They were discarded because the mother plants turned out to be less extreme than originally supposed at the moment of selection. A study of these groups separately disclosed no significant correlation in (a) ($r = -0{,}0207$). In (b) r had the value $+0{,}1828$, t^2 being 3,90 and P just slightly larger than 0,05. In group (c) there was no significant correlation, r being $+0{,}0205$, but in group (d) r reached the value $+0{,}3279$ with a t^2 of 4,577 and a P intermediate between 0,05 and 0,02.

In the 196 progenies of open-pollinated »Östgöta gråråg» plants the correlation between male and female fertility was found to be much more pronounced than in »Stålråg». As before, the average values of the progenies were used as variates. The value of r was found to be $+0{,}4483$, t^2 being 47,0 and P much smaller than 0,001. The variates showed the following distribution:

Per cent good pollen:	70 — 80	80 — 82,5	82,5 — 85,0	85,0 — 87,5	87,5 — 90,0	90,0 — 92,5
Average percentage of seed setting:	47,5	49,2	53,2	52,7	65,0	64,7
Number of progenies:	7	18	29	28	56	58

This material also comprised different subgroups just as in »Stålråg». In this case a significant positive correlation was found in group (a) (mother plants taken without selection). This group comprised 110 progenies and had an r value of $+0{,}3714$ ($t^2 = 16{,}16$ and

TABLE 3. *Correlation between percentage of good pollen and percentage of seed setting.*

Category number	Variety and year	n	r	Pollen fertility		Seed setting		Product of mean squares
				$\bar{x}$	mean square	$\bar{x}$	mean square	
1	Stålråg, 1937, »the long row»	98	+ 0,3873***	75,41	317,4	45,20	430,9	136768
2	» , » , »the short rows»	75	+ 0,1215	87,53	189,4	74,60	309,3	58581
3	» , 1938, »3—59 + 10—51»	119	+ 0,2880**	85,76	64,7	80,97	102,2	6612
4	» , » , field plot	102	+ 0,2156*	90,98	36,2	82,35	100,8	3649
5	Östgöta gråråg, 1938, field plot	103	+ 0,4165***	87,52	89,6	66,36	370,7	33215
6	Stålråg, 1938 —253, plants	613	+ 0,1576***	83,21	100,7	81,87	160,4	16152
7	» , » , progenies	86	+ 0,3654***	84,94	27,5	81,16	66,7	1834
8	» , 1938 —254, plants	521	— 0,1346**	88,40	57,9	77,92	64,2	3717
9	» , » , progenies	70	+ 0,0158	90,43	11,8	77,64	33,3	393
10	» , 1939, progenies, total	331	+ 0,1506**	87,32	11,8	79,58	30,7	362
11	» , » , » , Group a	118	— 0,0207	88,05	8,4	80,93	17,4	146
12	» , » , » , » b	115	+ 0,1828	87,77	11,4	80,93	22,6	258
13	» , » , » , » c	42	+ 0,0205	88,26	10,5	75,63	23,1	243
14	» , » , » , » d	40	+ 0,3279*	85,81	19,5	76,12	70,5	1375
15	Östgöta gråråg, 1939, total	196	+ 0,4483***	87,25	15,7	59,31	174,8	2744
16	» , » , Group a	103	+ 0,3714***	88,36	14,8	63,13	128,3	1899
17	» , » , » b	36	+ 0,0254	88,12	7,6	67,92	77,6	590
18	» , » , » c	50	+ 0,0077	85,35	18,2	45,90	79,0	1438

$P < 0{,}001$). In the offspring of 36 mother plants with especially good seed setting (Group b) no significant correlation could be established ($r = + 0{,}0254$). The same result was again met with in the offspring of 50 mother plants with an especially poor seed setting ($r = + 0{,}0077$). As usual in this material the average values of the progenies were used as variates.

The data reported above are somewhat conflicting, and sound conclusions can only be drawn from a comparison between the categories that are really comparable. All correlation coefficients obtained are summarized in Table 3. This table also includes all the average values of seed setting and pollen fertility as well as the corresponding mean squares. It also contains the products of the mean squares for seed setting and pollen percentage. This product roughly indicates the combined degree of variation of male and female fertility.

Of the 18 categories in Table 3, 7 (Nos. 1—6 and 8) concern the correlation between percentage of seed setting and percentage of good pollen in individual plants. Six of these categories show positive coefficients of correlation, r ranging from $+ 0{,}1215$ to $+ 0{,}4165$. In three of these (Nos. 1, 5, 6) the corresponding P values are smaller than 0,001, in one case (No. 3) P is only slightly larger than 0,001, in the fifth case (No. 4) P is intermediate between 0,05 and 0,02, in the sixth case (No. 2) P is larger than 0,05. In one single case r was found to be negative (No. 8), having the value $- 0{,}1346$ and a corresponding P intermediate between 0,01 and 0,001. In spite of this exceptional case it may be concluded that *in population plants of rye the percentage of good pollen and the percentage of seed setting after open pollination generally show a positive correlation.* The degree of this correlation, however, may be influenced by environmental conditions and is different in different rye varieties. Generally the r values tend to be high in material showing a strong variation and are low and insignificant in categories showing a low degree of variation.

Categories 1 and 2 in Table 3 were raised from the same seed material and hence the difference in strength of the correlation ($r = + 0{,}3873$ and $+ 0{,}1215$ respectively) is exclusively caused by the environmental differences. As mentioned above (p. 522), the plants of Category 1 were later and less well developed than those of Category 2. This difference is reflected by a greater variation and lower average values in Category 1 as compared with Category 2. The products of the variances are 136768 and 58581 respectively. The average values are as low as 75,41 and 45,20 in Category 1, the corresponding values of

Category 2 being 87,53 and 74,60. Thus, in the latter category the variation is rather onesided, most of the variates being close to their maximum values. In Category 1, on the contrary, the variation is more normal, and, hence, the correlation may become more marked.

A similar, though less extreme situation is met with in Categories 3 and 4. In both these cases the material is again ordinary »Stålråg», but the two groups were grown in different fields and had been sown with a different density. This may be responsible for the somewhat lower average values (85,76 and 80,97) in Category 3 than in Category 4 (average values 90,98 and 82,35 respectively). The former category is more variable than the latter one, the products of the mean squares being 6612 and 3649 respectively. Again the greater variation is associated with a higher degree of correlation, the *r* values being + 0,2880 and + 0,2156 respectively.

Category 5, field plot of »Östgöta gråråg», represents a genotypically quite different material, characterized by a low degree of seed setting, a pronounced degree of variation and a high *r* value. This group of material is directly comparable to Category 4, the field plot of »Stålråg» which was growing alongside the plot of »Östgöta gråråg». The average percentage of seed setting was 66,36 in the »Östgöta gråråg» plot, 82,35 per cent in »Stålråg». Pollen fertility was also somewhat lower in the former variety than in the latter one, the values being 87,52 and 90,98 respectively. The products of the mean squares of seed setting and pollen fertility were 33215 in »Östgöta gråråg» and only 3649 in »Stålråg». As before, the highest *r* value is found in the most variable material, the coefficient of correlation being + 0,4165 in »Östgöta gråråg» and + 0,2156 in »Stålråg».

Categories 6 and 8 (Table 3) represent the daughter plants of open-pollinated mother plants in Categories 1 and 2 respectively. This material of »Stålråg» was grown in the same field and, as far as possible, under the same conditions. Nevertheless there is a clear difference, the *r* value in Category 6 being + 0,1576 and that of Category 8 being — 0,1346. In the former category the variation is rather pronounced, in the latter category much smaller, the products of the mean squares being 16152 and 3717 respectively.

A contributing cause to this difference may be a slight degree of inbreeding in Category 6. In this case the mother plants flowered some days later than the mother plants of Category 8 and, thus, the pollen supply may have been scarce, especially as the plants were growing in a long row. This suspicion is supported by the low percentage of

seed setting among these mother plants (45,20 per cent). Hence some degree of self-fertilization may have occurred and may be responsible for the greater variation in Category 6 than in Category 8. In such a case fertility in Category 6 might be expected to be lower than in Category 8. This is true of the pollen fertility, the average values being 83,21 and 88,40 respectively. As regards the percentages of seed setting, however, the situation is reversed, Category 6 having a higher average value (81,87) than Category 8 (77,92).

The remaining categories in Table 3 do not represent individual plants but average values of the small progenies from open-pollinated mother plants. Anyhow, it is of interest to study the occurrence of correlations between male and female fertility in this material. Categories 7 and 9 represent the same material as Categories 6 and 8. As may be seen in Table 3, the use of the average values of the progenies as variates has naturally resulted in a lower degree of variation. The product of the mean squares for pollen fertility and seed setting in Category 6 is 16152 but only 1834 in Category 7. The corresponding products of Categories 8 and 9 are 3717 and 393 respectively. The *r* value of Category 7 is relatively high (+ 0,3654) and quite significant in contrast to the *r* value of Category 9 (+ 0,0158).

Significant positive correlations between the average male and female fertility were also observed in other categories of both rye varieties studied. In the material of »Stålråg» and »Östgöta gråråg» grown in 1939 only the average values of the different progenies were available for correlation studies. Considering first the material of »Stålråg», it is peculiar that significant correlations were only obtained in Category 10 (the whole material) and Category 14, which represents progenies raised from mother plants with an especially low degree of seed setting. A rather pronounced indication of a positive correlation was also met with in Category 12, which represents one of the groups from unselected mother plants. The high *r* value in Category 14 (+ 0,3279) coincides with a high degree of variation, the product of the mean squares being 1375. In the other groups of the »Stålråg» material in question the product ranged from 146 to 258. Thus, a selection of »bad» mother plants has increased the variation in the new population thus arising. This offers better opportunities to reveal the correlation between male and female fertility than is the case in the original material or in progenies selected in the other direction.

In the »Östgöta gråråg» progenies of 1939 another situation was met with. The total material (Category 15) shows a pronounced

positive correlation between male and female fertility, *r* having the value + 0,4483. Variation is great especially as regards the seed setting, the product of the mean squares being 2744. Of the three groups composing this total material Group *a* (Category 16) is derived from unselected mother plants. It has a high *r* value (+ 0,3714) and also a rather high product of the mean squares (1899). Groups *b* and *c* (Categories 17 and 18), on the contrary, do not show any correlation at all and their mean square products have lower values. These groups represent the progenies of mother plants with especially high and low degrees of seed setting. In »Stålråg» the progenies of plants with an especially poor seed setting showed a higher degree of correlation than the other categories. Here in »Östgöta gråråg» the result is evidently just the opposite. This paradox may probably be explained in the following way: In »Stålråg», which is a high-yielding variety, produced by a long period of selection, the average fertility is high and near the maximal one. »Östgöta gråråg» is a more primitive variety, showing a greater amount of variation and a lower average fertility especially as regards the percentage of seed setting. In »Stålråg» a selection in the minus direction will result in an increased variation, in »Östgöta gråråg», on the contrary, selection in the minus as well as the plus direction will diminish the degree of variation. Owing to the pronounced connection between degree of variation and numerical value of *r*, a minus selection in »Stålråg» will lead to an increased correlation between male and female fertility, whereas in »Östgöta gråråg» the opposite result will be obtained.

III. CORRELATION BETWEEN VIGOUR AND FERTILITY.

In most cases there was no correlation between vigour and fertility. However, in the »long row» of »Stålråg» grown in 1937 such correlations were observed and found to be significant. Thus, studying the correlation between plant height and percentage of seed setting an *r* value of + 0,3504 was obtained, t^2 being 13,30 and $P < 0{,}001$. The distribution of the variates may be given as follows:

Plant height in cm.:	20 — 60	— 80	— 100	— 130
Average percentage of seed setting:	29,0	41,5	49,0	52,5
Number of plants:	10	24	47	16

In the same material there was also a significant positive correlation between plant height and pollen fertility. In this case *r* had the

value + 0,2259, t^2 being 6,29 and P intermediate between 0,02 and 0,01. The variates showed the following distribution:

Plant height in cm.:	20 — 60	60 — 80	80 — 100	100 — 130
Percentage of good pollen:	67,4	74,1	78,1	78,8
Number of plants:	17	32	54	16

The same correlations were studied in the »short rows» of »Stålråg» growing in the same field. The result was quite negative and, thus, male as well as female fertility in this case were quite independent of plant height. Obviously, this difference is due to the fact that the »long row» was rather markedly modified owing to a late sowing in the preceding autumn. Plant height in this material ranged from 20 to 130 cm. with an average of 80,71 cm. In the »short rows» plant height ranged from 40 to 150 cm. with an average of 104,27. The difference between the two means is significant. As reported above (p. 522), there was also a significant difference in pollen fertility between the »long row» and the »short rows», the former category being more sterile than the latter.

In the next generation, represented by the plots 1938—253 and 1938—254 (progenies from the »low row» and the »short rows» respectively), the possible occurrence of correlations between vigour and fertility was closely studied. The average pollen fertility and seed setting on the one hand were correlated in all possible combinations with plant height and number of tillers on the other hand. Without going into details it may suffice to say that not a single significant correlation was obtained. Thus, in normally developed population plants of rye there is no correlation between vigour and fertility. The case in the »long row» of »Stålråg», studied in 1937, is clearly exceptional and due to the poor average development of this material. Anyhow, it is obvious that special environmental conditions may modify not only the vegetative development but also the degree of fertility.

IV. THE INHERITANCE OF MALE AND FEMALE STERILITY.

By comparing the degree of fertility of mother plants and offspring information should be obtained as to the causes of the variation in fertility observed in the rye populations. Data of this kind were obtained from the progenies studied in 1938 and 1939. These progenies were all

raised from open-pollinated mother plants. Progenies after isolation might be expected to give more precise results, but owing to the pronounced self-sterility in rye and the marked effects of inbreeding the problem could not be studied in this way. However, even after open pollination a correlation between mother plants and progenies may be expected, if the causes of the partial sterility are genotypic and not purely environmental. Such correlations were indeed observed.

1. FEMALE STERILITY.

In 1938 a total of 155 progenies from open-pollinated mother plants of »Stålråg» were studied and their average percentage of seed setting correlated with the degree of seed setting in the mother plant. The material consisted of two groups, 85 progenies being derived from the »long row» and 70 progenies from the »short rows» of 1937 (cf. p. 521). In both groups of material a significant positive correlation was established. In the offspring of the »long row» r was found to have the value $+ 0{,}3364$ with a t^2 of $10{,}59$ and a P intermediate between $0{,}01$ and $0{,}001$. The distribution of the variates was the following:

Percentage of seed setting in the mother plants:	10 — 30	30 — 40	40 — 50	50 — 60	60 — 70	70 — 90
Average percentage of seed setting in the progenies:	76,4	78,3	80,7	83,5	85,6	83,0
Number of progenies:	14	9	23	13	16	10

In the »short rows» the seed setting was less variable than in the »long row». Nevertheless, a quite significant positive correlation between mother plants and offspring was obtained, r having the value $+ 0{,}4314$, t^2 being $15{,}55$ and $P < 0{,}001$. In this case the distribution of the variates had the following appearance:

Percentage of seed setting in the mother plants:	30 — 50	50 — 70	70 — 80	80 — 90	90 — 100
Average percentage of seed setting in the progenies:	71,7	74,3	78,2	79,2	80,8
Number of progenies:	3	14	22	19	12

Further data from »Stålråg» were obtained in the following year, 1939. The total material comprised 332 progenies, and again a significant though rather weak positive correlation was obtained, r having the

value + 0,2341, t^2 19,13 and $P < 0{,}001$. The variates showed the following distribution:

Percentage of seed setting in the mother plants:	10 — 50	— 60	— 70	— 80	— 90	— 100
Average percentage of seed setting in the progenies:	75,0	80,0	79,2	79,3	80,6	81.6
Number of progenies:	12	18	26	71	152	53

As described above (p. 530), this material comprised four different groups, *a*—*d*, of which *a* and *b* represented the progenies of unselected mother plants, *c* progenies of mother plants with an especially high degree of seed setting and *d* progenies of mother plants with an especially low degree of seed setting. The coefficients of correlation were calculated for these groups separately, the following results being obtained:

Group:	*r*	t^2	*P*
a (no selection)	+ 0,2166	5,76	$0{,}02 > P > 0{,}01$
b (» »)	+ 0,1165	1,54	$0{,}3 > P > 0{,}2$
c (plus selection)	— 0,0994	0,40	$0{,}6 > P > 0{,}5$
d (minus »)	+ 0,3595	5,64	$0{,}05 > P > 0{,}05$
a—*d*, total	+ 0,2341	19,13	$< 0{,}001$

The most pronounced correlation is evidently met with in Group *d*. If the materials of Groups *a* and *b* are combined, the resulting coefficient of correlation will be + 0,1721 with a t^2 of 7,05 and a *P* intermediate between 0,01 and 0,001.

In the other variety »Östgöta gråråg» the positive correlation between seed setting in mother plants and offspring seems to be stronger than in »Stålråg». Data are only available from 1939. This year the material of »Östgöta gråråg» comprised a total of 197 progenies. Comparing these progenies with their mother plants, an *r* value of + 0,5563 was obtained, t^2 being 87,40 and *P* much smaller than 0,001. The variates in this material showed the following distribution:

Percentage of seed setting in the mother plants:	10 — 30	— 40	— 50	— 60	— 70	— 80	— 100
Average percentage of seed setting in the progenies:	50,0	47,9	50,3	55,0	56,5	62,4	68,3
Number of progenies:	12	24	19	21	20	35	66

This total material comprised three groups, *a*, *b* and *c*, of which the first one represented the offspring of unselected mother plants, the second group the offspring of mother plants with an especially high degree of seed setting and the third group the offspring of mother plants with an especially low degree of seed setting. Calculations of *r* in these separate groups gave the following result:

Group:	*r*	t^2	*P*
a (no selection)	+ 0,4284	22,92	< 0,001
b (plus »)	+ 0,1180	0,50	> 0,2
c (minus »)	+ 0,1423	0,99	> 0,2
Total	+ 0,5563	87,40	< 0,001

Thus, the positive correlation is strong in the unselected material but weak and insignificant in the two groups of selected material.

In Table 4 the data concerning the correlation between the seed setting of mother plants and offspring are summarized. Just as in Table 3, the average values and mean squares of the different categories are also given as well as the products of the mean squares. The main result to be seen in the table is the general occurrence of a positive correlation between the female fertility of mother plants and progenies raised after open pollination. Ten of the eleven *r* values are positive and several of them quite significant. The single negative *r* value is not significant. Considering the unselected categories (1, 2, 3, 4, 8), it is rather probable that the correlation is stronger in »Östgöta gråråg» than in »Stålråg». However, more material of the former variety is needed before this conclusion is quite safe.

The effect of selection on the magnitude of *r* is much the same as was the case in the correlation between male and female fertility. Thus, Category 6, representing a minus selection in »Stålråg», shows a greater *r* value than Category 5 (plus selection) and Categories 3 and 4 (no selection). The greater *r* in Category 6 is correlated to an increased degree of variation not only in the mother plants but also in the offspring. Therefore, the product of the mean squares in this category is as high as 18615, while the corresponding values in the other comparable categories of »Stålråg» range between 575 and 2573.

In »Östgöta gråråg» the opposite result was obtained just as in the case of the correlation between male and female fertility. The highest *r* value for the correlation between the seed setting of mothers and offspring was obtained in Category 8, representing unselected material.

TABLE 4. *Correlation between percentage of seed setting in the mother plants and average percentage of seed setting in the offspring.*

Category number	Variety and year	n	r	Average percentage of seed setting in		Mean squares of		Product of mean squares
				mother plants	offspring	mother plants	offspring	
1	Stålråg, 1938 —253	85	+ 0,3364**	49,12	81,35	322,1	71,1	22901
2	» , » —254	70	+ 0,4314***	76,57	77,86	198,9	32,3	6424
3	» , 1939, Group a (no selection) ...	119	+ 0,2166*	81,05	81,39	102,9	25,0	2573
4	» , » , » b (» ») ...	114	+ 0,1165	81,84	81,58	104,0	24,3	2527
5	» , » , » c (plus ») ...	42	— 0,0994	87,86	76,67	20,4	28,2	575
6	» , » , » d (minus ») ...	40	+ 0,3595*	59,05	76,75	228,4	81,5	18615
7	» , » , total	332	+ 0,2341***	79,58	80,12	170,5	36,5	6223
8	Östgöta gråråg, 1939, Group a (no selection)	104	+ 0,4284***	66,35	62,88	376,8	133,3	50227
9	» , » , » b (plus »)	36	+ 0,1180	86,39	68,06	12,3	101,8	1252
10	» , » , » c (minus »)	50	+ 0,1423	44,80	46,20	218,3	96,5	21066
11	» , » , total	197	+ 0,5563***	64,70	59,31	446,8	182,8	81675

In the Categories 9 and 10, representing plus and minus selection respectively, the *r* values are much lower. Again these categories show a lower degree of variation, not only in the mother plants but also in the offspring. The product of the mean squares is 50227 in the unselected material 1252 and 21066 in the plus and minus material.

The total material of »Östgöta gråråg» (Category 11) has a still higher *r* value and a still higher variation than the unselected material (Category 8). This is due to the fact that the average seed setting of Categories 9 and 10 has been strongly influenced by the selection. In the unselected material the average percentage of seed setting was 66,35 in the mother plants and 62,88 in the offspring (Table 4). In Category 9, representing the plus selection, mother plants with an average of 86,39 per cent gave an offspring with 68,06 per cent seed setting. In Category 10, representing the minus selection, the effect of the selection was still stronger, mother plants with an average of 44,80 giving an offspring with 46,20 per cent seed setting. In »Stålråg» the offspring of the minus plants (Category 6) also has a lower average value (76,75) than the offspring of the unselected mother plants (81,39 and 81,58). For unknown reasons, however, the plus selection in this variety entirely failed, the offspring in this category (No. 5) having a surprisingly low value (76,67).

2. MALE STERILITY.

The inheritance of different degrees of pollen sterility was examined in the same way as the female sterility, i. e. by studying the correlation between the percentage of good pollen in the mother plants and the average values of the offspring, derived from open pollination of the mother plants. In »Stålråg» the data from 1937—1938 represent the progenies derived from the »long row» and the »short rows» in 1937. The average values of the progenies are based on counts in about 10 plants per progeny.

The plot 1938—253, representing the offspring of the »long row», comprised 82 progenies. In this material positive correlation between pollen fertility of mother plants and offspring was obtained, *r* having the value + 0,2724, t^2 6,4117 and *P* being intermediate between 0,02 and 0,01. The variates were distributed in the following way:

Per cent good pollen in the mother plants:	20 — 50	70	80	90 — 100
Average percentage of good pollen in the progenies:	81,5	84,6	88,6	85,7	86,6
Number of progenies:	5	17	21	22	16

The plot 1938—254 comprised 71 progenies, derived from plants in the »short rows» of 1937. Also in this material r had a positive value, + 0,2041, but *per se* this correlation is not significant, t^2 being 3,00 and P intermediate between 0,1 and 0,05. The variates showed the following distribution:

Per cent good pollen in the mother plants:	10 — 80	85	90	95	100
Average percentage of good pollen in the progenies:	89,4	88,9	90,4	90,6	90,1
Number of progenies:	8	7	17	22	17

In 1939 the inheritance of partial pollen sterility could again be studied in two plots of »Stålråg» derived from unselected mother plants. These plots correspond to Categories 11 and 12 in Table 3 and to Categories 3 and 4 in Table 4. Comparing the progenies in the first plot (Group *a*) with their mother plants, r had the value + 0,0590. This correlation is not significant. The same calculation for the second plot (Group *b*) gave a higher value of r, + 0,2079. In this case t^2 was 5,06 and P intermediate between 0,05 and 0,02. The variates in this case showed the following distribution:

Per cent good pollen in the mother plants:	70 — 80	90	100
Average percentage of good pollen in the progenies:	82,4	85,6	87,0
Number of progenies:	6	31	77

Since Group *a* as well as Group *b* represents ordinary »Stålråg» from unselected mothers, the data of both groups may be combined. In this total group the r value will be + 0,1213, t^2 3,4464 and P larger than 0,05. Groups *c* and *d* from selected mother plants are absent in the present connection, as pollen fertility was not studied in these mother plants.

For the same reason the inheritance of partial pollen sterility in »Östgöta gråråg» could only be studied in Group *a* (Category 16, Table 3 and Category 8 in Table 4) which was derived from unselected mother plants. The result was not very striking, r having the value + 0,1440, t^2 being 2,82 and P larger than 0,05.

The total data obtained concerning the transmission of partial pollen sterility to the progenies of open-pollinated mother plants are summarized in Table 5.

According to the table all the coefficients of correlation have a positive value. Therefore, though only two of the r values show some

degree of significance, it is highly probable that in rye populations in general there is a positive correlation between degree of pollen fertility in mother plants and offspring. This correlation, however, is probably somewhat weaker than the corresponding correlation on the female side. This is again connected with a lower average degree of variation in the pollen than in the ovules. This is evident from a comparison of the mean squares and products of mean squares of Tables 4 and 5. If the corresponding categories are compared, the values relating to the seed setting are generally higher than those relating to pollen fertility. Only »Stålråg» 1939, Group *a*, is exceptional in this respect. In »Stålråg» 1938—253 and —254 the average male and female fertility in the progenies were determined on the same number of plants. In »Stålråg» 1939, on the contrary, the average values for female fertility are based on all the plants available (generally between 20 and 30 per progeny), whereas the male fertility is only based on counts from 5 plants. This will tend to increase the calculated degree of variation in pollen fertility more than the variation on the female side. As, nevertheless, seed setting was generally found to be more variable than the pollen fertility this emphasizes still more that the

TABLE 5. *Correlation between percentage of good pollen in the mother plants and average percentage of good pollen in the offspring.*

Category number	Variety and year	n	r	Average percentages of good pollen in		Mean squares of		Product of mean squares
				mother plants	offspring	mother plants	offspring	
1	Stålråg, 1938 —253	82	+0,2724*	75,67	85,06	265,8	26,7	7097
2	» » —254	71	+0,2041	87,90	90,18	187,3	17,0	3184
3	» » 1939, Group a	119	+0,0590	85,42	87,52	104,9	27,5	2885
4	» » » b	114	+0,2079*	91,23	86,40	34,3	19,2	659
5	» » , Groups a+b.....	233	+0,1213	81,82	86,97	21,8	23,7	517
6	Östgöta gråråg, 1939, Group a	103	+0,1440	87,72	88,79	86,7	47,3	4101

real variation is stronger on the female side than in the pollen. This difference seems to be much more pronounced in »Östgöta gråråg» than in »Stålråg».

V. DISCUSSION.

The occurrence of an incomplete seed setting in the ears of rye is a phenomenon well-known to farmers and practical breeders. As rye is a cross-fertilizer, this partial sterility has generally been supposed to be caused by incomplete pollination owing to unfavourable weather conditions at the time of flowering. In a few cases, however, evidence has been obtained that this incomplete seed setting may have special genotypical causes. In 1900 v. LOCHOW (cited from ROEMER, 1939) reported positive results from selection work in rye with the aim of decreasing the degree of »Schartigkeit». He also convinced himself as to the genotypical causes of the partial sterility by observations on the progeny of a single plant with an especially bad setting. Among a total of 56 daughter plants, 34 were highly sterile like the mother, 12 plants showed a weak degree of sterility and only 10 plants had a rather normal seed setting. Neighbouring plants, descending from normally fertile mother plants, all showed a good seed setting. In a paper from 1906, written in Swedish, LJUNG has reported quite interesting data concerning the occurrence of incomplete seed setting in rye. Starting from a plant with bad seed setting, LJUNG repeated a minus selection in several years and thus obtained a relatively constant strain in which only about half of the flowers set seed. He also observed that Probsteier-rye is heterogeneous as to the degree of seed setting and that selection in this material may lead to improvements. As a general experience LJUNG states that the degree of seed setting is of prime importance from the practical point of view and that this character is not only influenced by environmental conditions but may also be changed by selection.

In the important paper on rye by HERIBERT-NILSSON (1916) the main problem is the occurrence and genetical basis of self-sterility and self-fertility in the populations. However, a few observations concerning the occurrence of real sterility are also given (l. c., pp. 40—41). Thus, in F_2 of the crosses »Brattingsborg» × »Petkus» and »Brattingsborg» × »Heinrich» a few quite sterile plants were obtained and also some individuals with a very low degree of seed setting. A special strain of »Brattingsborg» also showed a high frequency of sterile plants, and

in this case, too, a continuous series of plants showing different degrees of failure of seed setting.

Besides the cases cited above, some other data may be mentioned which show that seed setting in rye populations is more or less reduced. In a paper chiefly dealing with the effects of isolation on seed setting ULRICH (1902) also reports the percentage of seed setting in open-pollinated ears of different rye varieties. The rather extensive material comprised three different series, the following percentage values being obtained:

Variety:	*Series 1*	*Series 2*	*Series 3*	*Average*
Petkus	81,65	79,00	74,09	78,25
Probsteier	59,97	64,62	59,19	61,26
Schlanstedter	77,60	76,26	81,95	78,60

In American rye varieties LEITH and SHANDS (1938) find that only 60 to 80 % of the total number of flowers in the head set seed in the field. This reduction in seed setting is ascribed to seasonal variations as well as to heritable differences. In a previous paper LEITH (1925) reports that during a 3-year study 33,2 % of 29760 flowers observed did not set seed under field conditions. PETERSON (1934) also finds that rye grown under ordinary field conditions shows considerable unfruitfulness, usually about one-third of the florets failing to set seed.

According to the data reported in the present paper, seed setting in the two Swedish varieties »Stålråg» and »Östgöta gråråg» shows a similar reduction. As may be seen in Tables 3—4, the percentage of seed setting in the former variety is generally about 80, in the latter variety about 65, this difference being significant. The higher value in »Stålråg» may certainly be ascribed to a long period of selection. In ULRICH's material, mentioned above, the variety Probsteier is evidently more sterile than the other two varieties. This is in good accordance with LJUNG's statement that Probsteier-rye is heterogeneous as to the degree of seed setting.

In my own material selection in a plus as well as a minus direction had very obvious effects in »Östgöta gråråg», whereas in »Stålråg» a similar selection gave less clear results. The nature of the partial sterility was further elucidated by correlating the percentage of seed setting in mother plants and offspring. Altogether 684 progenies were raised for that purpose. The main result was the demonstration of significant positive correlations in both varieties, the numerical value of the coefficients most probably being higher in »Östgöta gråråg» than

in »Stålråg». Thus, it is rather clear that the population plants are genotypically different with regard to genes causing different degrees of partial sterility. It is probable that these differences represent continuous gradations. Evidently, such gradations still occur in the highly selected and rather fertile variety »Stålråg».

So far, the occurrence in rye of a partial sterility on the male side as well seems to have been completely overlooked. The only reference to pollen sterility in rye populations prior to my paper in 1939 seems to be a statement by FRIMMEL and BARANEK (1935) that »auch Fälle von männlicher Impotenz nachgewiesen sind». This, however, probably refers to the occasional occurrence of plants or heads with aborted, non-dehiscing anthers. Such plants were also met with in the present material, but they are much less frequent than plants with normal anthers, containing a more or less bad pollen. The percentage of good pollen varies considerably from plant to plant and may also show a certain intra-plant variation. The inter-plant variation, however, was found to be significantly greater than the intra-plant variation. This result, in itself, suggests that the variation in pollen fertility has genotypical causes. This was further demonstrated by the general occurrence of positive correlations between the percentage of good pollen in mother plants and progenies (Table 5). These correlations are weak, it is true, but this may, indeed, be expected, firstly because the progenies were raised from open-pollinated mother plants, secondly because the average values of the progenies were based on values from a rather low number of plants per progeny.

Further evidence concerning the genotypical basis of the partial sterility in ordinary rye plants was obtained from studies of the correlation between male and female sterility. The general occurrence of positive correlations between percentage of seed setting and percentage of good pollen is evident from Table 3. As before, the highest coefficients of correlation were found in the most variable material, but in »Stålråg», too, the occurrence of such a correlation is beyond question.

The fertility variation in rye populations is probably not unique but typical of cross-fertilizing plant species in general. Several other allogamous species have been shown to behave in the same way at least as regards the occurrence of partial pollen sterility (MÜNTZING, 1939, Table 1). Later data in *Alopecurus myosuroides* (JOHNSSON, 1944, Table 3), diploid *Solanum* species (LAMM, 1944, p. 107), and in red clover (WEXELSEN, 1945, Table 9) point in the same direction. In the

last-mentioned species, however, partial pollen sterility was only met with in the cultivated material and not in a sample of wild clover.

The causes of the partial sterility occurring in the populations of rye and other allogamous plant species have been discussed previously (MÜNTZING, 1938, 1939, 1943 a, 1945; MÜNTZING and PRAKKEN, 1941). In these papers it was pointed out that the homozygous biotypes in autogamous species are stable products of selection, which are vital in the haplo- as well as the diplo-phase. The strong recombination in the allogamous species, on the other hand, will rather frequently give rise to unbalanced products, this being the probable cause of the sterility occurring. Structural chromosome deviations are the most frequent causes of sterility in higher plants and it is known that even quite small structural alterations are sufficient to kill the pollen grains receiving such an aberrant constitution. The embryo sacs also are sensitive to the same deviations, though in a smaller degree than the pollen. Cytological studies in population rye have demonstrated that cytological aberrations really occur in this species and that they are not rare. Therefore, it is not far-fetched to assume that sterility in rye and other allogamous plant species is largely due to structural chromosome variation. In such a case the sterility may be haplontic, i. e. involving the death of special classes of pollen grains and embryo sacs, which are killed by their own lethal constitution. The sterility may also be diplontic and be caused by the fact that certain plants represent a more or less pronounced quantitative deviation from the average constitution, the typical idiogram of the species. Finally, the ordinary gene recombinations may of course also influence the degree of seed setting and the development of the reproductive organs on the male side. Such genic influences may perhaps account for the fact that seed setting in »Östgöta gråråg» is much lower than in »Stålråg», though the amount of pollen sterility is almost the same in both varieties (cf. Tables 3 and 5).

Further information concerning the causal connections between heterozygosity, chromosomal aberrations and fertility may probably be obtained from cytogenetic studies of inbred rye lines and their hybrids. Thus, for instance, in studies of single and double crosses of inbred lines (MÜNTZING, 1943 b) significant differences in fertility were observed in different F_1 combinations. This was true of pollen fertility as well as seed setting and must be ascribed to genotypical differences between the parent lines. Such differences, whether structural or genic,

are evidently also responsible for the fertility variation in ordinary rye populations.

VI. SUMMARY.

(1) The occurrence and inheritance of partial sterility in the rye varieties »Stålråg» and »Östgöta gråråg» were studied. The occurrence of partially pollen sterile plants is a universal phenomenon in rye populations. The percentage of good pollen may be rather variable in the same plant and is influenced by environmental differences. Nevertheless, the inter-plant variation was found to be significantly greater than the intra-plant variation.

(2) Differences in seed setting in open-pollinated population plants of rye may either be caused by genotypical or environmental factors. The percentage of seed setting in »Östgöta gråråg» is much lower than in »Stålråg».

(3) In population plants of rye the percentage of good pollen and the percentage of seed setting after open pollination generally show a positive correlation. The degree of this correlation may be influenced by environmental conditions and is different in different rye varieties. Generally the *r* values tend to be high in material showing a strong variation and are low and insignificant in categories showing a low degree of variation.

(4) Generally there is a positive correlation between the percentage of seed setting in population plants and the average per cent of seed setting in their progenies, raised after open pollination. Ten of eleven *r* values based on a total of 684 progenies were positive and several of them quite significant.

(5) Studies on pollen sterility in the same material also revealed a general occurrence of positive correlations between mother plants and offspring. This correlation, however, is probably somewhat weaker than the corresponding correlation on the female side.

(6) The general conclusion is drawn that population plants of rye are genotypically different with regard to factors causing different degrees of partial sterility. It is probable that these differences represent continuous gradations. Such gradations still occur in the highly selected and rather fertile variety »Stålråg». The fertility variation in rye populations is probably not unique but typical of allogamous plant species in general.

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ABSTRACTS — KURZE MITTEILUNGEN

ÅKE GUSTAFSSON: The relationship of *Calamagrostis neglecta* and *lapponica* in Siberia, as interpreted by NILSSON-EHLE.

In a recent paper NYGREN (1946) has proved that the Scandinavian forms of *Calamagrostis purpurea*, *lapponica* and *chalybaea* (»*obtusata*») are apomictic, reproducing by means of diplospory and parthenogenesis. His extensive studies on natural populations indicate that these three species are related to the amphimictic species *C. canescens*, *neglecta* and *arundinacea* and may have evolved from them after crossing processes of various kinds or, quite simply, after an increase in chromosome number.

NYGREN (l. c., p. 207) refers to a paper by S. ALMQVIST, the Scandinavian expert of critical plant genera, in which the latter points out that, to judge from its properties, *C. purpurea* can be regarded as a hybrid population of *C. canescens* and *epigeios*. Such a hybrid was then synthesized by NYGREN and its appearance argued in favour of the interpretation mentioned.

An apparently correct view, however, has also been expressed concerning the mutual position of *C. lapponica* and *neglecta*. During the Swedish expedition to Siberia in search of S. A. ANDRÉE (1898—1899) NILSSON-EHLE gathered a large material of phanerogams and cryptogams, now deposited at the Museum of Natural History in Stockholm. In a treatise »Den subarktiska Lenafloran» (The subarctic Lena flora), written for his final university examination (1899), NILSSON-EHLE described his findings (unpublished). He treated the Siberian *Calamagrostis* material in detail, partially in collaboration with S. ALMQVIST. In his collections two specimens from meadows around the affluents of the Lena at Tigija and Bulkur were denoted as »*C. lapponica* × *neglecta*?».

NILSSON-EHLE writes (translated from Swedish):

»The form from Tigija closely resembles *C. neglecta*. With regard to the size of spikelets and pollen grains as well as the length of hairs on the lemmas it occupies an obviously middle position between *C. lapponica* and *neglecta*. All pollen grains lack contents. The latter property would justify a hybrid interpretation, were it not for the fact that in *C. lapponica* itself the pollen always is quite useless. The specimen from Bulkur is even more similar to *C. lapponica* but has somewhat smaller spikelets. If these two forms are not hybrids, but true middle forms, *C. lapponica and neglecta ought not to be distinguished as different species but should be treated as varieties of one and the same species.*»

The outstanding fact of the data briefly quoted here is that in the nineties NILSSON-EHLE put forward a similar view as did (independently of him) NYGREN in 1946 concerning the mutual position of *C. lapponica* and *neglecta*, and that he distinctly referred to the pollen qualities as distinguishing the populations. Besides their historical interest his notes on Siberian material indicate the general correctness of NYGREN's interpretation, chiefly based upon Scandinavian collections.

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GÖSTA VON ROSEN: Chromosome determination in root-tips and leaves by the rapid orcein method.

In a previous paper (VON ROSEN, 1946) the author described a rapid method for assorting polyploid plant material. This method is only suitable for counting chromosomes in leaves and is based on MAYER's report on the advantages of colchicine for this purpose.

However, it ought to be of advantage for the plant-breeder to have at his disposal a rapid method for the estimation of chromosome numbers in root-tips as well. In working out the principles given below for such a method the author adapted it in the first place to root-tips, forced on the first year's roots of *Beta vulgaris*. But the method can also be employed with advantage on ordinary root-tips from plantlets. Moreover, it also seems to be better and more reliable for leaf material than the method published earlier.

The author wishes to stress, however, that a rapid method of the type presented here, which is based on a squeezing out of chromosome accumulations, preferably at the metaphase stage, into artificial »equatorial plates» in which the chromosomes are more or less clearly separated, is in the first place intended to serve plant-breeders as a means of more rapidly assorting polyploid materials into their respective polyploidy groups. In work on series of 10 to 30 samples this simplified method may be estimated to ensure a daily capacity about five times greater than the customary paraffin-gentian method.

When an exact count of the chromosome complement is required, the ordinary slower method is recommended until more experience has been gained.

The method has been elaborated at the Hilleshög Beet Breeding Institute, Landskrona, of the Swedish Sugar Company.

It is a pleasure to the author to take this opportunity of tendering to Dr. A. LEVAN and Miss M. PALM of the Cyto-Genetic Laboratory, Svalöf, his cordial thanks for the great interest shown by them in discussions and experiments during the elaboration of this method.

Root-tips.

Fixation. — If the material cannot be manipulated in a fresh state, the roots are fixed in a mixture of 2 parts of abs. alc. + 1 part of chloroform (note: alkaline fixation), in which they ought to be kept at least 3 hours before being stained. They may be kept in this mixture a couple of weeks or longer.

A rapid method of the kind described here demands a greater supply of suitable divisions for judgment than the paraffin-gentian method, and therefore the root-tips must be in an intense period of division when fixation is undertaken.

Good roots on plantlets can be forced without difficulty. If it is wished to facilitate the counting work on plants with long chromosomes, the chromo-

somes can be induced to contract, for which purpose the current method of allowing the plants to stand cool during the night before sampling is the simplest.

When it is desired to force rootlets on fully grown beets, this may be done at any time in the winter. However, a storage time of at least 1 1/2 months should elapse between harvesting and forcing. The roots are placed between layers of wet sacking in an ordinary greenhouse kept at about + 10° to + 15° C. and are kept constantly damp by sprinkling with temperated water. When the root-tips have become about 5—10 mm. long, the roots are transferred, wrapped in their sacking, to a heated chamber having a saturated atmosphere at + 20° to + 22° C. A chamber of this kind is easy to arrange. In a closed room of small size, e. g. a wash-house, a chamber is constructed of a small wooden frame entirely covered with jute sacks, the latter being frequently sprayed with water. The chamber is fitted with an electric heating-unit or a hot-water radiator and 100 per cent saturation is ensured by placing water-containers both over the radiator and on the floor. For control purposes a thermometer should be placed among the beets in the chamber, as too high a temperature is directly injurious.

The roots are kept in this heating chamber for 4—6 hours.

In order to contract the chromosomes the sacking is sprayed with cold water for a couple of minutes and the roots are allowed to cool for 2—3 hours in a room having a temperature of + 3° to + 5° C., after which sampling is carried out as described above.

If chromosome contraction is not desirable, fixation can be immediately undertaken after the beets have been taken out of the heating-chamber.

Preparing for staining. — Fresh material that is to be judged immediately need not be fixed but treated direct as described below.

Fixed roots are after-treated and fresh roots are fixed in a mixture of 1 part of abs. alc. + 2 parts of normal butyl alcohol for 4—5 minutes. This solution was proposed and tried out in experiments by Miss PALM. Long treatment causes the chromosomes to swell more than is desirable. The chromosomes are less sensitive to the 2 : 1 mixture.

Staining. — Three to five root-tips are placed on an object-glass and arranged with the root-tips close together and concentrated to the zones of division by cutting away the stretching parts. This operation must not take so long a time that the roots lose their dampness. If it is found necessary to add an extra drop of alc.-butyl, this should be sucked off before staining. One drop — in exceptional cases two — of 2 % orcein (prepared according to DARLINGTON and LA COUR, 1942, p. 110) is added. The material is carefully pulped in the solution and a cover-glass is placed over. Contact between material and fluid may be conveniently effected by means of light taps on the cover-glass. Depending on the nature of the material, staining is effected between 5 and 15 minutes without heating. (Beet roots require 10 minutes.) With a staining time of 15 minutes or more the plasm is usually stained more or less deeply. After cold staining the object-glass is placed on a *hot* copper-plate and the cover-glass is gently tapped. Heating time 30—60 seconds. It is to be noted that the preparation must not boil. The hot preparation is placed on a firm support (e. g. a thick glass plate), and, with light pressings

round the edge of the cover-glass, the superabundant staining fluid is sucked out with thin filter-paper, e. g. Grycksbo No. 10. This operation must not take so long a time that the preparation gets cold (maximum time: 10 seconds). The filter-paper is left in position and pressure is immediately exercised for 10—15 seconds by passing a rubber roller to and fro both lengthwise and crosswise. At first the pressure should be mild, harder towards the end. The preparation is then ready for judgment.

For rapid microscopical work a light-strong magnifying system is required. The best contrasts in the picture are obtained when the light is filtered with a Zeiss trichromic filter. This requires a strong lamp. A pointolite lamp the light of which is concentrated through lenses seems to be preferable. A matt filter is of course necessary. If a highly heat-generating lamp is used, it is suitable to insert a Zeiss heat-filter under the diaphragm. More rapid microscopical work is obtained by a careful adjustment of the magnifying system to the purpose in view. It is waste of time to work with too low or too high a magnification, when zones of division are being studied. If a microscope is used in which the ocular tube magnifies 1,5 times, the most effective magnifying system is an objective 20 × and an ocular 7 ×. For chromosomes having the size of those in, for instance, *Beta* and *Pisum* the chromosome counting itself is done with the aid of an objective 40 × and an ocular 7 × or 15—20 ×. Should a higher magnification be required, the thinness of the preparation will allow of an immersion objective being used without difficulty.

Leaves.

Fixation. — The above method also admits excellently of being used for the determination of chromosomes in leaves. For this the very youngest leaves which have not yet emerged out of the bud-cluster are the best (in peas: the leaflets or stipules that have not grown out of the bud-cluster; in beets: young leaves that have just separated from the floral cluster at the time when this can be macroscopically observed, i. e. when the shooter is 2—3 decimetres high). As a rule, of course, on each plant those shoot-tips should be taken where the division rhythm is most intense.

In species with small chromosomes, e. g. *Beta vulgaris*, the chromosomes should be immediately scattered at mitosis with a weak solution of colchicine by disturbing the spindle and simultaneously contracting them (see VON ROSEN, 1946). Depending on the material, the concentration of the colchicine solution is selected between 0,05 % and 0,5% , which gives the optimum result. The chromosomes show a typical *c*-mitosis. Treatment should last 1—3 hours; with longer treatment the leaf-tissues blacken.

Leaves that are not to be stored before being judged do not require fixing.

To preserve the material for some time it is fixed as described above for root-tips.

Staining. — Pre-treatment with a solution of alcohol-butyl alcohol has not proved suitable for the types of plants hitherto tested by the author.

The leaf is put direct on an object-glass and thick midribs are cut away. In plant types with paired leaves, e. g. pea, the leaf-surface are separated. The leaf is not pulped but only divided cautiously into large (and perhaps small)

pieces. Orcein solution is added as described above and a cover glass is placed over. Here, too, contact between material and fluid may be conveniently effected by means of light taps on the cover-glass.

It has been found most advantageous to employ direct heat. The heating is carried on 1—3 minutes, i. e. longer than for root-tips, or so long that it can be seen that the leaf-fragments, which before staining gleam brightly in the red fluid, have stained to a fairly even pink. The absorption of fluid and squeezing out are effected as in the case of root-tips.

Thick and more or less coriaceous leaves (e. g., *Fragaria*, *Helianthus*, *Lilium*, etc.) or leaves containing fatty substances (e. g., *Beta*, *Betula*, *Malus*, etc.) should be stained with a mixture: 1 part 2 % orcein + 1 part chloroform. (The mixture to be shaken; see VON ROSEN, 1946.) The leaves should be carefully pulped in the solution and a cover-glass placed over. Contact between material and fluid must be effected.

To avoid formation of the extremely poisonous phosgene gas, that is produced when chloroform is exposed to an open flame, the preparation must in no case be heated over a spirit flame. The most suitable method is to warm the copper-plate on a weak electric heating plate.

Buds.

Finally, it may be mentioned that this staining method may very well also be used for the study of meiosis in the P.M.C's.

Either direct staining is used or else fixation in alcohol-chloroform solution. No pre-treatment is necessary in alcohol-butyl alcohol.

The stamens are dissected out on the object-glass and cold staining is done for 5—10 minutes in the way already described, followed by a very short heating before the squeezing-out operation is started. The latter should in this case be carried out less powerfully than in the case of roots and leaves.

Hilleshög, March 16th, 1946.

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